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Variabilidade genética em populações de *Pilosocereus catingicola* (Gürke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi (Cactaceae)

Genetic variability in populations of *Pilosocereus catingicola* (Gürke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi (Cactaceae)

Variabilidad genética en poblaciones de *Pilosocereus catingicola* (Gürke) Byles & Rowley subsp. *salvadorensis* (Werderm.) Zappi (Cactaceae)

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Resumo

Estudos sobre diversidade genética de cactáceas são importantes para elucidar acontecimentos evolutivos e características ecológicas de populações vulneráveis a erosão genética. O objetivo do estudo foi avaliar a diversidade genética entre indivíduos de *Pilosocereus catingicola* subsp. *salvadorensis* ocorrentes em três populações localizada na Caatinga do Agreste paraibano, utilizando-se marcadores RAPD. Para a extração do DNA foram utilizados tecidos do parênquima e do cladódio do caule dos indivíduos, pelo método CTAB 2% e amplificado utilizando-se 05 iniciadores. As marcas obtidas foram convertidas em uma matriz binária, a partir da qual foi construída a matriz de dissimilaridade genética usando o complemento aritmético do coeficiente de Jaccard e a construção do dendrograma, pelo o método UPGMA. Todos os marcadores testados amplificaram, sendo 82,4% *locus* polimórficos e 10,6% monomórficos para a população de Arara; 84,4% polimórficos e 15,6% monomórficos para a população de Areial, a população de Boa Vista não apresentou monomorfismo, resultando em 100% de polimorfismo. As médias de heterosigose observada (0,372, 0,492 e 0,135) evidenciam que há divergências genéticas dentro das populações. Os *primers* utilizados foram eficazes na identificação de polimorfismo na espécie. As populações avaliadas neste estudo apresentaram alta diversidade genética, tanto dentro quanto entre população, originando quinze grupos entre os 120 indivíduos a partir da análise de agrupamento hierárquico UPGMA, sendo possível discriminar as populações.

Palavras-chave: Facheiro; DNA; Polimorfismo.

Abstract

Studies on genetic diversity of cacti are important to elucidate evolutionary events and ecological characteristics of populations vulnerable to genetic erosion. The objective of this study was to evaluate the genetic diversity among individuals of *Pilosocereus catingicola* subsp. *salvadorensis* occurring in three populations located in the Caatinga do Agreste region of Paraiba, using RAPD markers. For the extraction of DNA, tissues of the parenchyma and cladodium of the stem of the individuals were used by the 2% CTAB method and amplified using 05 primers. The obtained marks were converted into a binary matrix, from which the matrix of genetic dissimilarity was constructed using the arithmetic complement of the Jaccard coefficient and the construction of the dendrogram, by the UPGMA method. All markers tested amplified, being 82.4% polymorphic locus and 10.6% monomorphic for the Arara population; 84.4% polymorphic and 15.6% monomorphic for the Areial population, the Boa Vista population did not show monomorphism, resulting in 100% polymorphism. The

averages of heterosigose observed (0.372, 0.492 and 0.135) show that there are genetic divergences within the populations. The primers used were effective in identifying polymorphism in the species. The populations evaluated in this study showed high genetic diversity, both within and between the population, originating fifteen groups among the 120 individuals from the UPGMA hierarchical grouping analysis, and it was possible to discriminate the populations.

Keywords: Facheiro; DNA; Polymorphism.

Resumen

Los estudios sobre la diversidad genética de los cactus son importantes para dilucidar los eventos evolutivos y las características ecológicas de las poblaciones vulnerables a la erosión genética. El objetivo del estudio fue evaluar la diversidad genética entre individuos de *Pilosocereus catingicola* subsp. *salvadorensis* ocurrente en tres poblaciones ubicadas en la Caatinga de Agreste Paraibano utilizando marcadores RAPD. Para la extracción de tejidos de ADN del parénquima y el cladodo del tallo de los individuos, se utilizó el método CTAB 2% y se amplificó utilizando 05 cebadores. Las marcas obtenidas se convirtieron en una matriz binaria, a partir de la cual se construyó la matriz de disimilitud genética utilizando el complemento aritmético del coeficiente Jaccard y la construcción del dendrograma, utilizando el método UPGMA. Todos los marcadores probados amplificados, con un 82,4% de locus polimórfico y un 10,6% de monomórfico para la población de Arara; 84.4% polimórfico y 15.6% monomórfico para la población de Areial, la población de Boa Vista no mostró monomorfismo, resultando en un 100% de polimorfismo. Las medias de heterosigosis observadas (0.372, 0.492 y 0.135) muestran que existen diferencias genéticas dentro de las poblaciones. Los cebadores utilizados fueron efectivos para identificar el polimorfismo en la especie. Las poblaciones evaluadas en este estudio mostraron una gran diversidad genética, tanto dentro como entre poblaciones, dando lugar a quince grupos entre los 120 individuos del análisis de conglomerados jerárquicos de UPGMA, lo que permite discriminar las poblaciones.

Palabras clave: Facheiro; ADN; Polimorfismo.

1. Introduction

Among the diversity of cacti found in the Americas, there is the species *Pilosocereus catingicola* subsp. *salvadorensis*, with wide distribution in caatinga areas in the wild areas in

Paraíba, popularly known as “facheiro”. Information about the smell is still scarce in the literature, however, it is known that it is referenced for medicinal purposes, such as fuels, in ornamentation, and its fruit is usually consumed *fresh* or used in the manufacture of candies (Lucena et al., 2013).

The dominance or subdomain of Cactaceae species in the vegetation physiognomy of the Caatinga of Northeast Brazil, mainly of the genera *Cereus*, *Pilosocereus* and *Tacinga*, has been of a real importance in the maintenance of the local fauna (Rocha and Agra, 2002). However, there is still a deficit of information regarding cacti, mainly studies involving the genetic diversity of the natural population of the species, which could subsequently provide subsidies for genetic improvement, conservationist programs and the formation of germplasm banks.

In this context, studies with genetic diversity can elucidate events throughout life history and ecological characteristics which can help to identify groups of populations vulnerable to the responsible factors for the erosion of genetic diversity. However, studies that are available on cactus populations are still restricted to 30 of approximately 1,600 species, which were analyzed using different genetic markers, including allozymes, RAPDs and ISSRs (Clark-Tapia et al., 2005; Casas et al., 2007).

The advances in research on plant genetic diversity, in the contemporary era, have been intensified by means of technological innovations in genetics and molecular biology, which have made it possible through the technique of recombinant DNA, polymerase chain reaction and automatic DNA sequencing made possible the development of molecular markers capable of detecting genetic polymorphism directly in DNA, and it is used for identification, characterization and evaluation of plant genetic resources (Faleiro, 2007).

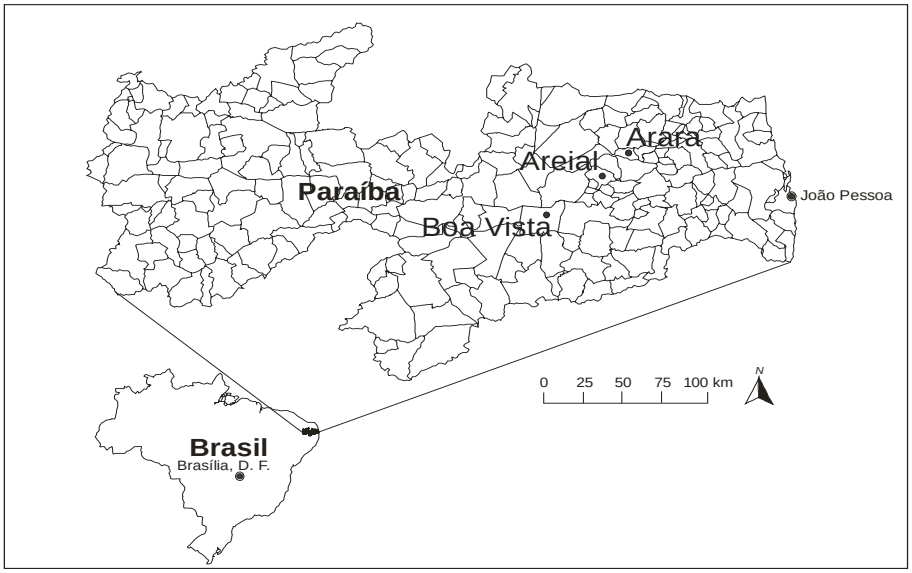
Currently, there is a considerable number of molecular markers, the choice of one or the other depending on the technological level and available financial resources. One is RAPD technique (*Random Amplified Polymorfhic* DNA) uses short arbitrary primers, which result in the amplification of several DNA products in the same PCR reaction, which are derived from a region of the genome that contains two segments homologous to the primers, on opposite strands of DNA and close enough for amplification to occur (Williams et al., 1990).

Given the above, the objective of the study was to conduct a study of genetic diversity among populations of *Pilosocereus catingicola* subsp. *salvadorensis* of occurrence in caatinga areas of wild areas in Paraíba using RAPD markers.

2. Material and Methods

The nature of this study was characterized as a field and laboratory research, as reported by Pereira et al. (2018). The study was carried out in three populations of *Pilosocereus catingicola* subsp. *salvadorensis*, located in different landscapes of forested Caatinga areas, in the municipalities of Arara, Areial and Boa Vista, PB (Figure 1).

Figure 1. Geographic location of the study areas and collection of plant material from *Pilosocereus catingicola* subsp. *salvadorensis* (Werderm.) Zappi for analysis in the different municipalities of wild areas in Paraíba, Brazil.



Source: elaborated by authors.

The municipality of Arara is located in the Mesoregion of wild areas in Paraíba and in the Microregion of Curimataú Ocidental, inserted in the geoenvironmental unit of the Borborema Plateau formed by massifs and high hills, with soil fertility varied between medium and high (CPRM, 2005a).

The municipality of Areial is located in the Mesoregion of the wild areas in Paraíba, in the Microregion of Esperança and is inserted in the geoenvironmental unit of Borborema Plateu, with formations of subcaducifolic and deciduous forests, slightly wavy soils with the presence of Planossols, strongly drained, moderately acidic and medium to high natural fertility (CPRM, 2005b).

The municipality of Boa Vista is located in the Mesoregion of wild areas in Paraíba, in the Microregion of Campina Grande and in Borborema Plateau, cariri from Paraíba,

geographically inserted in the Brazilian semiarid region, presenting medium to high soil fertility, covered by Caatinga vegetation formations where plant species pass through deciduous in the dry season (CPRM, 2005c).

The three areas were chosen because they present characteristics of Caatinga forest formations at different levels of ecological succession, and previously registered the natural occurrence of *Cactaceae* with high abundance of species of the the type: *Pilosocereus,* besides the need to investigate *in loco* the variability of edaphic attributes where the species occurs, in order to subsidize information about the ecology of the species in the region

Forty plants were selected randomly per area, totaling 120 plants, all georeferenced with the aid of a GARMIN ETREX® 30 GPS with Universal Transverse Mercator - UTM coordinates. Parts of the stem of each plant were collected and taken for molecular analysis at the Plant Biotechnology Laboratory of the Center for Agricultural Sciences - CCA, at the Federal University of Paraíba - UFPB, Campus II, in Areia.

DNA extraction was performed according to the protocol by Porebski et al. (1997) with modifications, using the 2% (CTAB) method, approximately 200g of medullary and cladodal tissue was used, weighed and macerated in liquid N2. DNA cleaning was carried out by means of RNA hydrolysis and quantified by electrophoresis in 0.8% agarose gel at 80 Volts for 30 minutes and photographed under UV light, on a Gel Logic 112 photo-documenter. amplification of PCR and electrophoresis, the protocol of Luna-Paez et al. (2007) with changes. The RAPD analyzes were performed with five primers (Table 1).

Table 1. RAPD initiators, using for access analysis of *Pilosocereus catingicola* subsp. *salvadorensis.*

Initiator	Sequence	Size
AL01	5´-CAT TCG AGC C-3´	10pb
AL04	5´-CCG CCC AAA C-3´	10pb
AL07	5´-AGC GAG CAA G-3	10pb
AL12	5´-TGG CCC TCA C-3´	10pb
AL17	5´-CCC AGC TGT G-3´	10pb

Source: elaborated by authors.

Through the products of the DNA amplification reactions in the gel, using (RAPD markers), a binary matrix was constructed, in which ("0") indicated absence and ("1"), the presence of bands and converted into a matrix of binary data from which genetic similarity

between individuals was estimated, generating a dissimilarity matrix by means of the arithmetic complement (1-c) of the Jaccard similarity coefficient (1908).

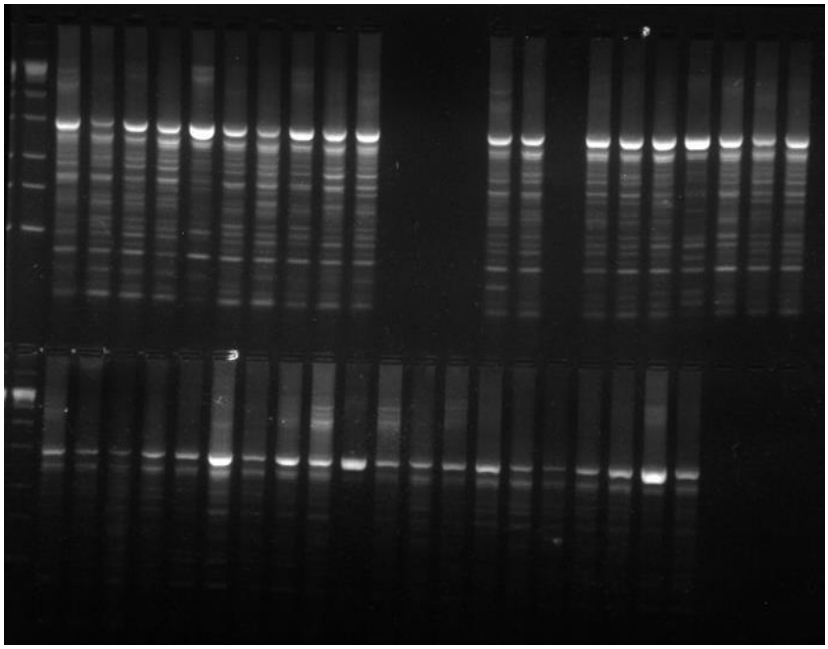
The genetic distance matrix was performed to perform the cluster analysis using the dendrogram, using the hierarchical clustering method UPGMA (*Unweighted Pair Group Method Arithmetic nean*). For the cut in the dendrogram, the criterion proposed by Mojema (1977) was adopted, which is based on the relative size of the levels of fusions (distances) in the dendrogram. The adjustment between the similarity matrix and the dendrogram was also calculated using the co-phenetic correlation coeficiente (r), according to Sokal and Rohlf (1962). All analyzes were performed using the Genes software (Cruz, 2006).

3. Results and Discussion

The protocol adopted by Porebski et al. (1997) with modifications, used in the extraction of DNA of *Pilosocereus catingicola* subsp. *salvadorensis*, proved efficient in the quantity and quality of the extracted DNA, providing a clean visualization of the DNA through the electrophoresis technique. Several tests were carried out for DNA extraction, samples of the medullary tissue, stood out due to the higher concentration of DNA, while the sample from the cladode did not have a good result, given the high concentration of hydrocolloids, which made DNA analysis unfeasible, due to the difficulty of isolating the DNA.

Amplification occurred in all five primers used in the three populations of facheiro, showing to be efficient for polymorphism detection in *Pilosocereus catingicola* subsp. *salvadorensis*, because even though it's a small amount (cinco *primers),* was sufficient to identify high levels of polymorphism between and interpopulation (Figure 2).

Figure 2. DNA amplification products generated with an AL12 primer in 40 genotypes of *Pilosocereus catingicola* subsp. *salvadorensis* of the population of Areial-PB, using the RAPD technique. MM - weight marker 1 kb Ludwig.



Source: elaborated by authors.

For the population of Arara, the RAPD analysis, of the five primers used, it was possible to generate a total of 1,492 fragments. 82.4% fragments were polymorphic and 10.6% monomorphic. And the *primer* AL04 was the one with the highest number of amplification and the highest proportion of polymorphic loci (359 bands), the *primer* AL17 was the one with the lowest amplification numbers, and even with the lowest numbers of amplified products, the *primers* AL01, AL07 and AL17 were the initiators that presented greater proportions 100% of *loci* polymorphic each (Table 2). The high level of polymorphism found shows that there is genetic diversity among the 40 individuals studied in the population of Arara.

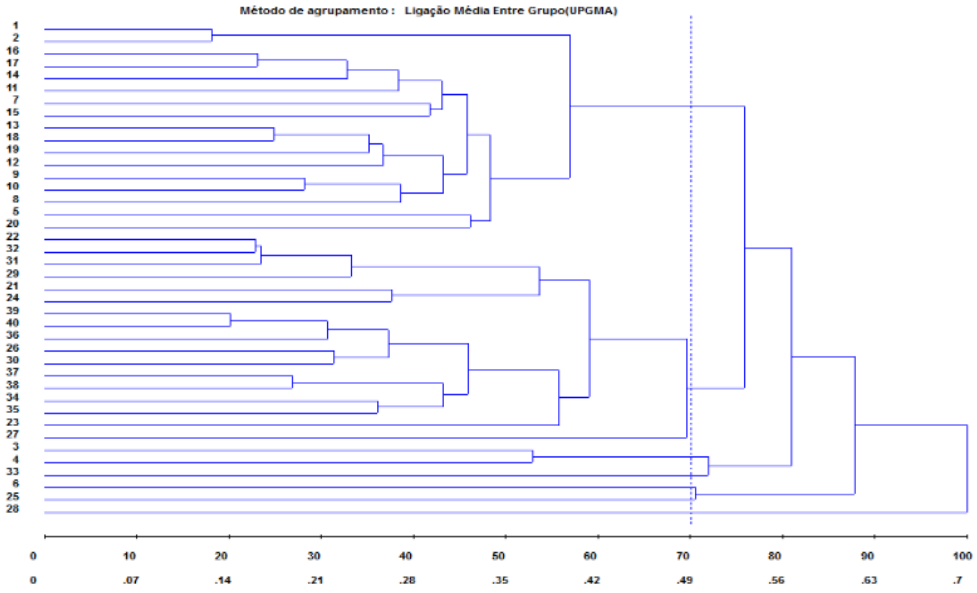
Table 2. Numbers of amplified fragments, polymorphic and monomorphic *loci* obtained for the 40 genotypes of *Pilosocereus catingicola* subsp. *salvadorensis* of the population of Arara-PB, using five RAPD primers.

Initiator (*Primer*)	Sequence (5'-3')	Number of loci amplified	Number of loci polymorphic	Number of loci monomorphic
AL 01	5´-CAT TCG AGC C-3´	263	263	0
AL 04	5´-CCG CCC AAA C-3´	478	359	119
AL 07	5´-AGC GAG CAA G-3´	253	253	0
AL 12	5´-TGG CCC TCA C-3´	291	252	39
AL 17	5´-CCC AGC TGT G-3´	207	207	0
Total		1.492	1.334	158

Source: elaborated by authors.

The cluster analysis, using the UPGMA method, carried out based on genetic distances, allowed to allocate the 40 individuals of *P. catingicola* subsp. *salvadorensis* in six groups of genetic similarity, the cutoff was made according to the criteria of Mojema (1977); (Figure 3).

Figure 3. Dendrogram resulting from the analysis of 40 genotypes of *Pilosocereus catingicola* subsp. *Salvadorensis* of the population of Arara-PB, obtained by the UPGMA method, based on the genetic dissimilarity matrix obtained by the arithmetic complement of the Jaccard similarity coefficients, using 5*primers* RAPD.



Source: elaborated by authors.

The similarity values allowed the individuals to be divided into seven groups, with group I and II are the most populous, both with 17 individuals and four distinct groups, indicating genetic kinship (Table 3).

Table 3. Grouping of individuals from *Pilosocereus catingicola* subsp. *salvadorensis* of the population of Arara-PB, using the UPGMA method based on the Jacard similarity coefficient.

Group	Individuals
I	1 2 16 17 14 11 07 15 13 18 19 12 9 10 8 5 20
II	22 32 31 29 21 24 39 40 36 26 30 37 38 34 35 23 27
III	3 4
IV	33
V	6
VI	25
VII	28

Source: elaborated by authors.

The groups that allocated a greater number of individuals were group I and group II. The third group is represented by individuals 03 and 04, the other groups (four) were formed individually, containing only one individual each, this implies that they are genetically distinct individuals with no degree of kinship, so they have not grouped themselves into any group. It is still important to note that 84% of the individuals were grouped in the first and second groups, noting that, although similar, they still have some genetic divergence.

The consistency of the clusters is evidenced by the co-phenetic correlation coefficient (CCC), (Table 4), which was obtained from the genetic distance matrix and the co-phenetic distance matrix, from the dendrogram. (r = 0.85 **), considered high and significant. Co-phenetic correlation coefficients less than 0.7 indicate inadequacy of the grouping method (ROHLF, 1970). 1.93% distortion and 13.91% stress were also obtained, considered good values according to the Kruskal scale (1964). The good representation of the dissimilarity matrix in the shape of the dendrogram is confirmed by the low distortion and stress level found.

Table 4. Coenetic correlation coefficient between the distance matrix and the UPGMA hierarchical clustering method, generated from the analysis of genetic diversity evaluated among the 40 genotypes of *pilosocereus catingiola* subsp. *salvadorensis* the population of Arara-PB using RAPD markers.

Cophenetic correlation (CCC):	0,8542
Distortion (%):	1,9352
Stress (%):	13,912

Source: elaborated by authors.

For the population of Areial, of the five primers used (Table 5), 84.4% loci were polymorphic and 15.6%, monomorphic, with primer AL04 having the highest number of amplified fragments. The *primer* AL07 presented a lower number of amplification. Even presenting the smallest number of amplified products, AL1, AL07 and AL17 were the *primes* which presented the highest proportions of polymorphic bands (100% each).

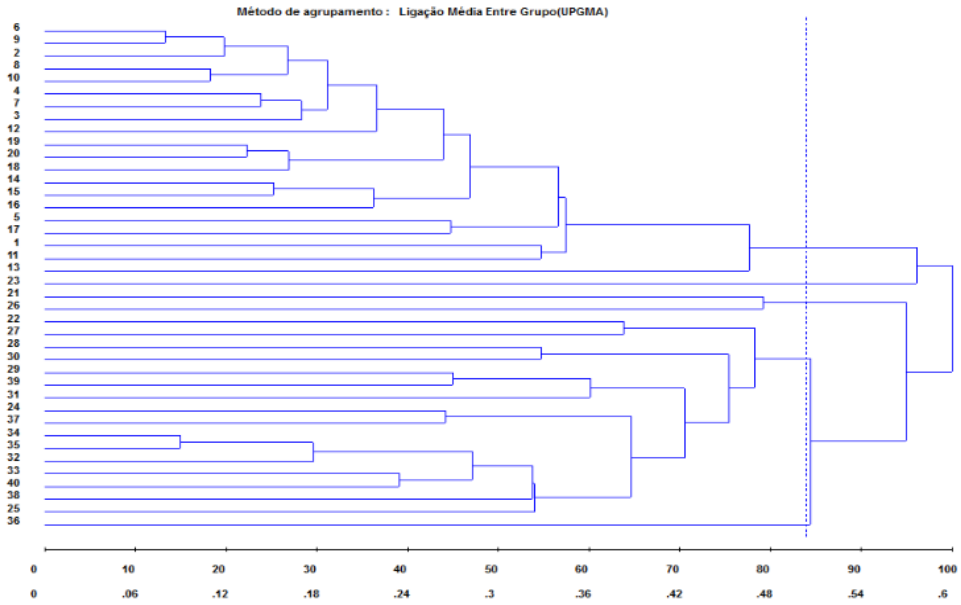
Table 5. Numbers of amplified fragments, polymorphic and monomorphic loci obtained for the 40 genotypes of *Pilosocereus catingicola* subsp. *salvadorensis* of the population of Areial-PB, using five RAPD primers.

Initiator (*Primer*)	Sequence (5'-3')	Number of amplified *loci*	Number of polymorphic loci	Number of monomorphic *loci*
AL 01	5´-CAT TCG AGC C-3´	310	310	0
AL 04	5´-CCG CCC AAA C-3´	516	399	117
AL 07	5´-AGC GAG CAA G-3´	238	238	0
AL 12	5´-TGG CCC TCA C-3´	399	246	156
AL 17	5´-CCC AGC TGT G-3´	297	297	0
Total		1.760	1.487	273

Source: elaborated by authors.

The cluster analysis (Figure 4), using the UPGMA method, performed based on genetic distances, allowed to allocate the 40 individuals of *P. catingicola* subsp. *salvadorensis* in five groups of genetic similarity, with group I and IV are the most populous, formed by 20 and 16 individuals respectively, indicating similarity between them, and 1 distinct group with only one individual, without degree of genetic kinship.

Figure 4. Dendrogram resulting from the analysis of 40 accesses of *P. catingicola* subsp. *salvadorensis* of the population of Areial-PB, obtained by the UPGMA method, based on the genetic dissimilarity data obtained by the arithmetic complement of the Jaccard similarity coefficients, using 5 *primer* RAPD.



Source: elaborated by authors.

The groups that allocated a greater number of individuals were groups I and group IV, although showing similarity between them, they have genetic divergences. The third group is represented by individual 23 and is characterized as a distinct group with no degree of genetic kinship, the greater the individuals are divergent, the lower the chance of kinship between them, thus representing a high level of intrapopulation genetic diversity (Table 6).

Table 6. Grouping of individuals from *Pilosocereus catingicola* subsp. *salvadorensis* of population of Arara-PB, using the UPGMA method based on the Jacard similarity coefficient.

Group	Individuals
I	6 9 2 8 10 4 7 3 12 19 20 18 14 15 16 5 17 2 11 13
II	23
III	21 26
IV	22 27 28 30 29 39 31 24 37 34 35 32 33 40 38 25
V	36 25

Source: elaborated by authors.

The group three and five allocated two individuals each, implying that they are genetically distinct individuals, without any degree of kinship, so they did not group themselves into any group. It is worth mentioning that 50% of the population's individuals were grouped in the first, noting that, although similar, they still have some genetic divergence.

The co-phenetic correlation coefficient (CCC) (Table 7), obtained from the genetic distance matrix and the co-phenetic distance matrix, from the dendrogram, was considered high (r = 0.89**) and significant, showing, thus, the consistency of the groupings. 1.82% distortion and 13.50% stress were also obtained, considered good values.

Table 7. Cophenetic correlation according to the molecular data (RAPD) evaluated in the 40 genotypes of *P. catingiola* subsp. *salvadorensis* of the population of Areial - PB.

Cophenetic correlation (CCC):	0,8949
Distortion (%):	1,823
Stress (%):	13,5016

Source: elaborated by authors.

The Boa Vista population showed greater genetic diversity with polymorphism in all analyzed *primers* (Table 8), with the primer AL12 generating the largest number of amplified products, while AL01 generating the lowest number of *loci*, all polymorphic (100%) characterizing it as being of high genetic diversity.

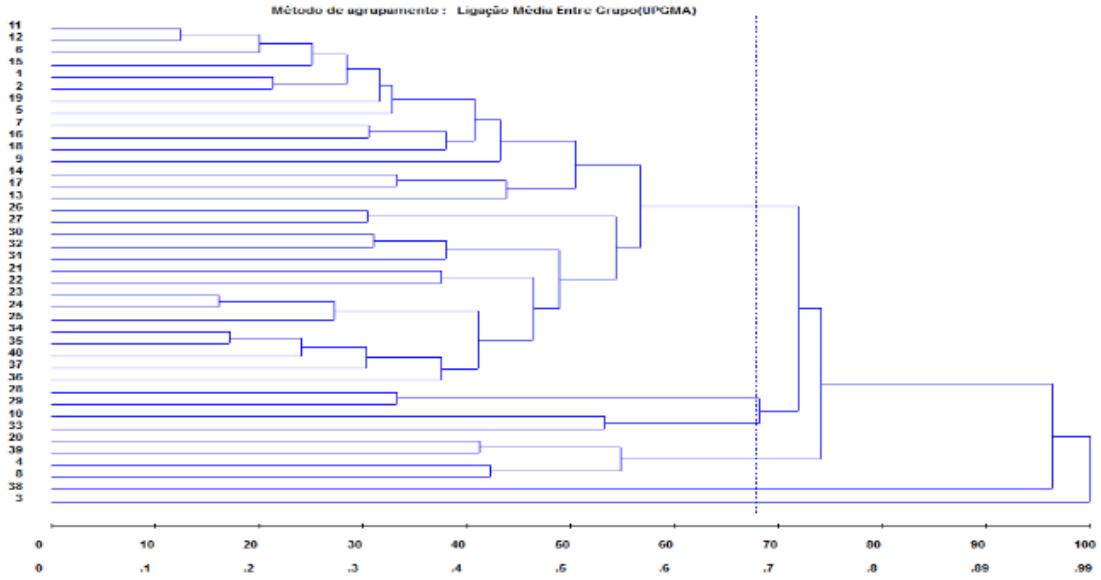
Table 8. Numbers of amplified *loci*, polymorphic and monomorphic loci obtained for the 40 genotypes of *P. catingicola* subsp. *salvadorensis* of population of Boa Vista-PB, using five RAPD primers.

Initiator (*Primer*)	Sequence (5'-3')	Number of amplified *loci*	Number of polymorphic *loci*	Number of monomorphic *loci*
AL 01	5´-CAT TCG AGC C-3´	99	99	0
AL 04	5´-CCG CCC AAA C-3´	261	261	0
AL 07	5´-AGC GAG CAA G-3´	245	245	0
AL 12	5´-TGG CCC TCA C-3´	263	263	0
AL 17	5´-CCC AGC TGT G-3´	123	123	0
Total		991	991	0

Source: elaborated by authors.

The cluster analysis, using the UPGMA method, carried out based on genetic distances, allowed to allocate the 40 individuals of *P. catingicola* subsp. *salvadorensis* in six groups of genetic similarity, with a greater similarity between individuals 11 and 12 (Figure 5).

Figure 5. Dendogram resulting from the analysis of 40 genotypes of *Pilosocereus catingicola* supsb. *salvadorensis* of the population of Boa Vista-PB, obtained by the UPGMA method, based on the genetic dissimilarity data obtained by the arithmetic complement of the Jaccard dissimilarity coefficients, using 5 *primer* RAPD.



Source: elaborated by authors.

Among the six groups formed (Table 9), the most populous group is group I, comprising 75% of the individuals, although similar, they still have some genetic divergence. This similarity may reflect the fragmentation process that the Caatinga ecosystem has been suffering, thus causing crosses between related individuals.

Several factors can contribute to unviable seeds and seedlings such as inbreeding, one of the main causes of the loss of genetic differentiation, especially in forest species, seeing that most of them have cross-breeding (Shimizu, 2007). Therefore, the knowledge of the genetic variability existing in populations that have undergone anthropic actions is of great relevance, aiming to contribute to the development of adequate management in these populations and the recovery of degraded environments (Rabbani et al., 2012).

Table 9. Grouping of individuals from *Pilosocereus catingicola* subsp. *salvadorensis* of the population of Boa Vista-PB using the UPGMA method based on Jacard's similarity.

Group	Individuals
I	11 12 6 15 1 2 19 5 7 16 18 9 14 17 13 26 27 30 32 31 21 22 23 24 25 34 35 40 37 36
II	28 29
III	10 33
IV	20 39 4 8
V	38
VI	3

Source: elaborated by authors.

The cohenetic correlation coefficient (CCC) (Table 10), obtained was considered high (r = 0.89 **) and significant, showing the consistency of the clusters. 1.76% distortion and 13.28% stress were also obtained, considered good values. The low distortion and stress level confirm the good representation of the dissimilarity matrix in the shape of the dendrogram. According to Cruz and Carneiro (2006), the higher the CCC, the lower the cluster distortion, with an agreement between the original dissimilarity values and those represented by the dendogram.

Table 10. Cophenetic correlation according to the molecular data (RAPD) evaluated in the 40 genotypes of *Pilosocereus catingiola* subsp. *salvadorensis* in the population of Boa Vista-PB.

Cophenetic correlation (CCC)	0,8931
Distortion (%):	1,7657
Stress (%):	13,2888

Source: elaborated by authors.

The data also allowed a study of genetic differentiation between and within populations where a high level of diversity was observed. The population of Boa Vista showed high levels of polymorphism (100%), this variation was also confirmed when the populations were evaluated together. The genetic diversity between populations of forest species from different successional groups is concentrated mainly within populations.

The interpopulation genetic diversity in forest species is quite variable, demonstrating

that the reproductive system, the dispersion of pollen and seeds, in addition to the successional stage, are determining factors of the population genetic structure (Fowler and Glienke, 2008). The high percentage of polymorphism identified in this study is of great importance for the adoption of conservation and management strategies in the analyzed natural populations, considering that human intervention in ecosystems, especially in their endemic species, results in the extraction of trees of greater vigor and better and better quality, leaving only those of inferior quality to transmit their genes to the next generations, in a dysgenic process that leads to the degradation of the remnants.

The analysis of the results allowed the observation of the inter and intraspecific genetic diversity between the accessions as well as the intergenerational distinction, as well as it concluded that the intraspecific genetic diversity is greater for the accesses of the population of Boa Vista with 100% polymorphic, that means to say that there is indeed a high degree of genetic diversity in this population.

Through cluster analysis, carried out based on genetic distances, the 120 individuals of *Pilosocereus catingicola* subsp. *salvadorensis* were allocated to fifteen groups of genetic similarity. It is possible to discriminate populations, that is, there were no groupings between individuals located in different areas, but there is a union of individuals according to their geographical distribution and the formation of distinct groups located in the same physical space, thus showing that there is a high genetic diversity without degrees of kinship between individuals in these populations (Table 11).

It is also worth noting that 85% of the individuals in the population of Arara were grouped in group three; 50% of individuals from the population of Areial in group seven and 77.5% of individuals from the population of Boa Vista in group one, it is also observed that the groups were formed by individuals from the same geographical area, which confirms a high level of diversity interpopulation.

Table 11. Grouping of individuals from *Pilosocereus catingicola* subsp. *salvadorensis* of the three populations; Arara, Areial and Boa Vista in the rough of Paraíba by the UPGMA method using Jacard's similarity data.

Group	Individuals
I	103 104 106 107 105 101 102 114 115 116 111 117 112 120, 110 113 87 89 91 92 81 86 82 99 95 85 93 98 96 97 94 90
II	3 4 33
III	1 2 16 17 14 11 7 15 13 18 19 12 9 10 8 5 20 22 32 31 29 21 24 39 40 36 26 30 37 38 34 35 23 27
IV	6 25
V	61 66
VI	62 67 68 70 69 79 71 64 77 74 75 72 73 80 78 65 76
VII	41 51 45 57 54 55 56 59 60 58 48 50 46 49 42 44 47 43 52 53
VIII	63
IX	108 109
X	28
XI	100 119
XII	84
XIII	88
XIV	118
XV	83

Source: elaborated by authors.

The high index of genetic diversity observed in this work may be related to several factors, such as population size, gene flow, pollination, natural selection and seed dispersal method and mainly to the reproductive system, seeing that the species reproduces by cross-pollination. The average heterosigosis observed in the three populations of Arara, Areial and Boa Vista, was (0.372; 0.492 and 0.1335), considered high when compared with the data found by Clark-Tapia et al., 2005), which was 0.158.

The co-phenetic correlation coefficients generated from the genetic distance matrices and the co-phenetic matrices showed a significant and high correlation between the dendrograms obtained from the accessions of the three studied populations, varying between 0.85 and 0.89, with low distortion and low stress, confirming a good representation of the dissimilarity matrices in the form of the dendrogram.

The stress presented by the three studied populations was low (13.91; 13.50 and

13.28), considered good according to the Kruskal scale (1964), the distortion was also considered low (1.82; 1, 93 and 1.76). The high co-phenetic correlation coefficients, the low levels of distortion and stress obtained in this study, confirm the good representation of the dissimilarity matrix in the shape of the dendrogram, and a greater, reliability in the results.

4. Conclusion

The medullary tissue of the facheiro is the most promising for extracting the DNA, providing a greater quantity of extracted DNA with good quality. In this sense, the use of this tissue appears to be the most promising for the extraction, isolation and preparation of samples for PCR in this species.

The primes used RAPD were efficient in identifying polymorphism of *Pilosocereus catingicola* subsp. *salvadorensis.*

The analysis of genetic diversity demonstrated a high level of polymorphism in populations of *Pilosocereus catingicola* subsp. *salvadorensis*, both within and between populations. It is suggested that other studies in this line of research be developed with other species of Cactaceae.

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