



ORIGINAL ARTICLE

Prospection and genetic diversity of bromeliad genera in fragmented areas

Prospecção e diversidade genética de gêneros de bromélias em áreas fragmentadas

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PALAVRAS-CHAVE

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ABSTRACT: The bromeliad family comprises approximately 3,000 species distributed in 58 genera and 3 subfamilies: Pitcairnioideae, Bromelioideae and Tillandsioideae. The increasing demand of the market for bromeliads has been responsible for increasing the production and sale of these plants. However, illegal harvesting is still dramatically reducing existing species. This fact, coupled with the devastation of Brazilian ecosystems, has caused irreparable losses in diversity. Thus, having a germplasm bank with conserved genetic material for future studies is very important. The use of molecular markers, together with the description of the vegetative and reproductive characters of a species, is an asset for studies of natural populations for conservation programs and assists in properly classifying plant species. The aim of this work was to characterize various genera of bromeliads by ISSR molecular markers and morphological characteristics. The samplings were carried out in four municipalities of the State of Sergipe (Itabaiana, Frei Paulo, Simão Dias and Poço Redondo), whose locations were mapped using GPS. Individuals were transferred and kept in a greenhouse, thereby comprising an Active Germplasm Bank (AGB). For the molecular analyses, young leaves were collected and used for DNA extraction. Eleven ISSR primers were used to characterize the prospected accessions. The data generated by the ISSR markers and morphological characteristics enabled the identification and separation of the bromeliad accessions of the AGB.

RESUMO: A família das bromélias tem cerca de três mil espécies, distribuídas em 58 gêneros e três subfamílias: Pitcairnioideae, Bromelioideae e Tillandsioideae. A crescente demanda do mercado tem sido responsável pelo aumento de produção e venda de bromélias. No entanto, a extração ilegal ainda está reduzindo drasticamente as espécies existentes. Este fato, juntamente com a devastação dos ecossistemas brasileiros, tem causado perdas irreparáveis na diversidade. Dessa forma, ter uma base com o material genético armazenado para estudos futuros é muito importante. O uso de marcadores moleculares aliado à descrição dos caracteres vegetativos e reprodutivos de uma espécie auxilia os estudos com populações naturais para programas de conservação e para aplicação na taxonomia das espécies vegetais. O objetivo deste trabalho foi caracterizar alguns gêneros de bromélias por ISSR (Inter Sequências Simples Repetidas), marcador molecular, e por características morfológicas. As coletas das plantas foram realizadas em quatro municípios do Estado de Sergipe (Itabaiana, Frei Paulo, Simão Dias e Poço Redondo), cujos locais foram mapeados com o uso de GPS. Os indivíduos foram transferidos e mantidos em uma estufa, compondo um Banco Ativo de Germoplasma (BAG). Para as análises moleculares, folhas jovens foram coletadas e utilizadas para a extração de DNA. Onze iniciadores ISSR foram utilizados para caracterizar os acessos prospectados. Os dados gerados pelos marcadores ISSR e morfológicos possibilitaram a identificação e a separação dos acessos do BAG de bromélias.

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1 Introduction

Bromeliads are plants of the Bromeliaceae family, which contains approximately 60 genera and 3,170 species (LUTHER, 2006). According to Nunes (1997), Pitcairnioideae, Tillandsioideae and Bromelioideae are the three subfamilies that represent the Bromeliaceae family. The subfamilies are divided according to the floral characteristics and the morphology of fruits and seeds. Bromeliads are perennial plants that possess a wide variety of forms, colors and sizes. They occur preferentially in tropical areas, extending from North America to South America, with one species occurring in Africa (BENZING, LUTHER; BENNETT, 2000).

The Atlantic Forest is one of the major centers of diversity of the Bromeliaceae family. It is also one of the biomes with the highest rate of destruction on the planet (HERINGER; MONTENEGRO, 2000), causing the fragmentation of native populations. Such a scenario has contributed to the loss of genetic diversity of several species. In concert with this, the Bromeliaceae family is widely used in ornamentation and landscape projects due to the diversity of colors of its inflorescence and its ability to remain showy for weeks, as well as for the beauty of its leaves. The combination of habitat destruction and harvesting for domestic use has resulted in a decline in natural populations and the loss of a great part of its genetic material.

Molecular tools are an asset for studies of natural populations for conservation programs (HAMRICK; GODT, 1989). One example of a useful molecular tool are the ISSR markers, which are valuable in studies of genetic mapping, population genetics and marker-assisted selection in plant breeding and feed (LACERDA; ACEDO; LEMOS-FILHO, 2001; PINHEIRO et al., 2012.). These markers are low in cost and provide an unlimited number of polymorphic fragments, covering almost the entire genome (FERREIRA; GRATTAPAGLIA, 1998; BERTONI et al., 2010.).

Information generated about the vegetative and reproductive characteristics enables better characterization of species, which has applications both in taxonomy and in conservation programs.

The aim of this work was to characterize various genera of bromeliads by ISSR molecular markers and their morphological characteristics.

2 Materials and Methods

The prospection and germplasm collection were carried out from August 2011 to February 2012 in four municipalities (Poço Redondo, Frei Paulo, Simão Dias and Itabaiana) of Sergipe State (Figure 1).

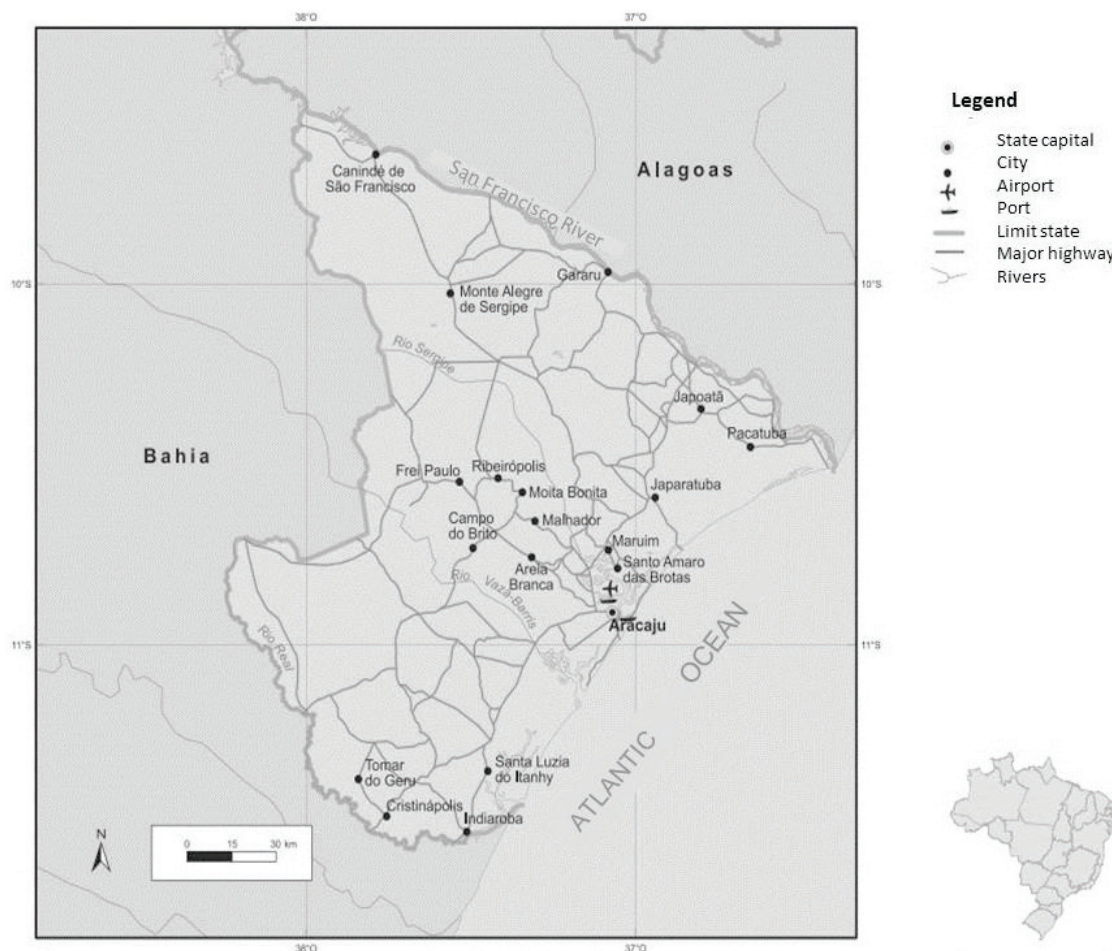


Figure 1. Map of the Sergipe State identifying prospected locations (<http://7a12.ibge.gov.br/images/7a12/estados/sergipe.pdf>).

The plants found in the expeditions were geo-referenced with a Global Positioning System (GPS, Garmin).

Seeds were collected when present in the prospected individuals and were then coded and used to create an Active Germplasm Bank (AGB) in a greenhouse at the Department of Agronomic Engineering, Universidade Federal de Sergipe (UFS). The substrate used was earthworm castings + coconut coir (1:1), and the size of the vessels was chosen according to the size of the individuals.

Species identification was performed in the herbarium (ASE) of the Universidade Federal de Sergipe (UFS), which is located in the Department of Biology of the Federal University of Sergipe. The parameters used to identify the species were primarily the leaf structures, such as the position of the prickles, the presence or absence of hair, the insertion of the sheath and characteristics of the inflorescence.

For genomic DNA extraction, the method of Doyle and Doyle (1991) was used. Analyses of Inter Simple Repeated Sequences (ISSR) were performed at the Central Laboratory of Seeds of the Universidade Federal de Lavras (UFLA). Eleven primers were used (Table 1). The total volume of the reaction was 12 µL containing 2 µL of the genomic DNA solution and 2 µL of each primer, together with a mixture composed of sterilized ultrapure water, 1X buffer [1.5 mM MgCl₂, 100 mM MgSO₄, 100 mM KCl, 80 mM (NH₂)₂SO₄], 100 mM Tris-HCl] (NeoTaq), 0.6 µL dNTPs (10 mM) and Taq polymerase (5 U. µL⁻¹). Next, the material was amplified in a thermocycler (Axygen). To visualize the fragments, amplification products were submitted to horizontal electrophoresis at a voltage of 100 V for 2 h and 30 min in a 1.5% agarose gel.

The electrophoretic profile of each ISSR primer gel was transformed into a binary matrix, with the presence of a fragment represented by 1, and the absence represented by 0. These binary data were used to estimate the similarities.

3 Results and Discussion

A total of 34 subsamples of prospected bromeliads were found in the municipalities of Poço Redondo (01), Frei Paulo (06), Simão Dias (08) and Itabaiana (19) in the Sergipe State. It was possible to form an AGB with the accessions. In total, three genera divided into two subfamilies were identified at

the Herbarium (ASE) of the Universidade Federal de Sergipe (Table 1).

The characteristics used by Dr. Graça Wanderley, Dr. Gardene Maria Sousa and Dr. Ana Paula de Nascimento to identify species are listed according to the related subfamily (Table 2).

A total of 370 DNA fragments were generated using 11 primers (Table 3), all polymorphic. Primer 842 produced the greatest number of fragments (50), and the ISSR 808 primer was the least productive (21).

In the clustering based on the multivariate analysis (PCA; Figure 2), the first two principal coordinates explain 24.64% of the total variability, and explaining 80% of the variance required 2.32 of the 0.78 detected eigenvalues. The variance explained by F1 and F2 was below 50%, but the results confirmed the differences between genera. The accession I_Amultiflora1 grouped with the accessions I_Amultiflora2 I_Amultiflora3 (black group), as both belong to the same subfamily (Bromelioideae) and present similar morphological characteristics. The accession I_Tpolistachia3 grouped with I_Tpolistachia1 (green group), as well as I_Vsp1 SD_Vsp3 (red group), which were collected in different municipalities. I_Tpolistachia4 grouped with I_Tpolistachia2 (blue group), which belong to the same subfamily (Tillandsioideae) and present similar morphological characteristics. The accession FP_Tpolistachia2 grouped with I_Vsp3 FP_Tpolistachia1 (yellow group), although these accessions belong to different species, *Tillandsia* and *Vriesea*, respectively (Figure 2).

This result can be explained by the literature, as the Tillandsioideae and Bromelioideae subfamilies have problems of circumscription of genera and species (BARFUSS et al., 2005). Despite the important contribution of many authors to the systematic classification of these two subfamilies (HORRES et al., 2000; BARFUSS et al., 2005), the systematic relationships remain very artificial within the family. Even after advances in the classification of subfamilies of Bromeliaceae, resulting in the formation of natural groups, the delimitation of genera has been widely discussed (GIVNISH et al., 2011), especially that of *Tillandsia*, which represents a paraphyletic genus.

Table 1. Bromeliads identified by accessions. Municipality, subfamily, genus and number of accessions.

Municipality	Subfamily	Genus	Number of subjects
Itabaiana	Bromelioideae	<i>Aechmea</i>	7
	Tillandsioideae	<i>Vriesea</i>	6
	Tillandsioideae	<i>Tillandsia</i>	6
	Bromelioideae	<i>Aechmea</i>	2
Simão Dias	Tillandsioideae	<i>Vriesea</i>	5
	Tillandsioideae	<i>Tillandsia</i>	1
Poço Redondo	Bromelioideae	<i>Aechmea</i>	1
Frei Paulo	Tillandsioideae	<i>Tillandsia</i>	6

Table 2. Vegetative and reproductive characteristics of the subfamilies of Bromeliaceae.

Subfamily	Part of the plant	Characteristic
Bromelioideae	Ovary	Infero
	Fruit	Bracteos
	Seed	Naked
	Leaf	Serrated margins
	Flower	Sessile
Tillandsioideae	Ovary	Supero
	Fruit	Capsular dehiscent
	Seed	Plumose appendixes
	Leaf	Presence of peltate trichomes
	Flower	Trimera

Table 3. ISSR primers, base sequence and number of obtained polymorphic/total fragments.

Primer	Sequence	Number of bases	Number of polymorphic fragments
01	ACA CAC ACA CAC ACA CT	17	26
808	AGA GAG AGA GAG AGA GC	17	21
810	GAG AGA GAG AGA GAG AT	17	23
812	GAG AGA GAG AGA GAG AA	17	29
827	ACA CAC ACA CAC ACA CG	17	32
834	AGA GAG AGA GAG AGA GYT	19	46
842	GAG AGA GAG AGA GAG AYG	19	50
855	ACA CAC ACA CAC ACA CYT	19	33
866	CTC CTC CTC CTC CTC CTC	18	27
888	BDB CAC ACA CAC ACA CA	23	40
891	HVH TGT GTG TGT GTG TG	23	43
Total			370

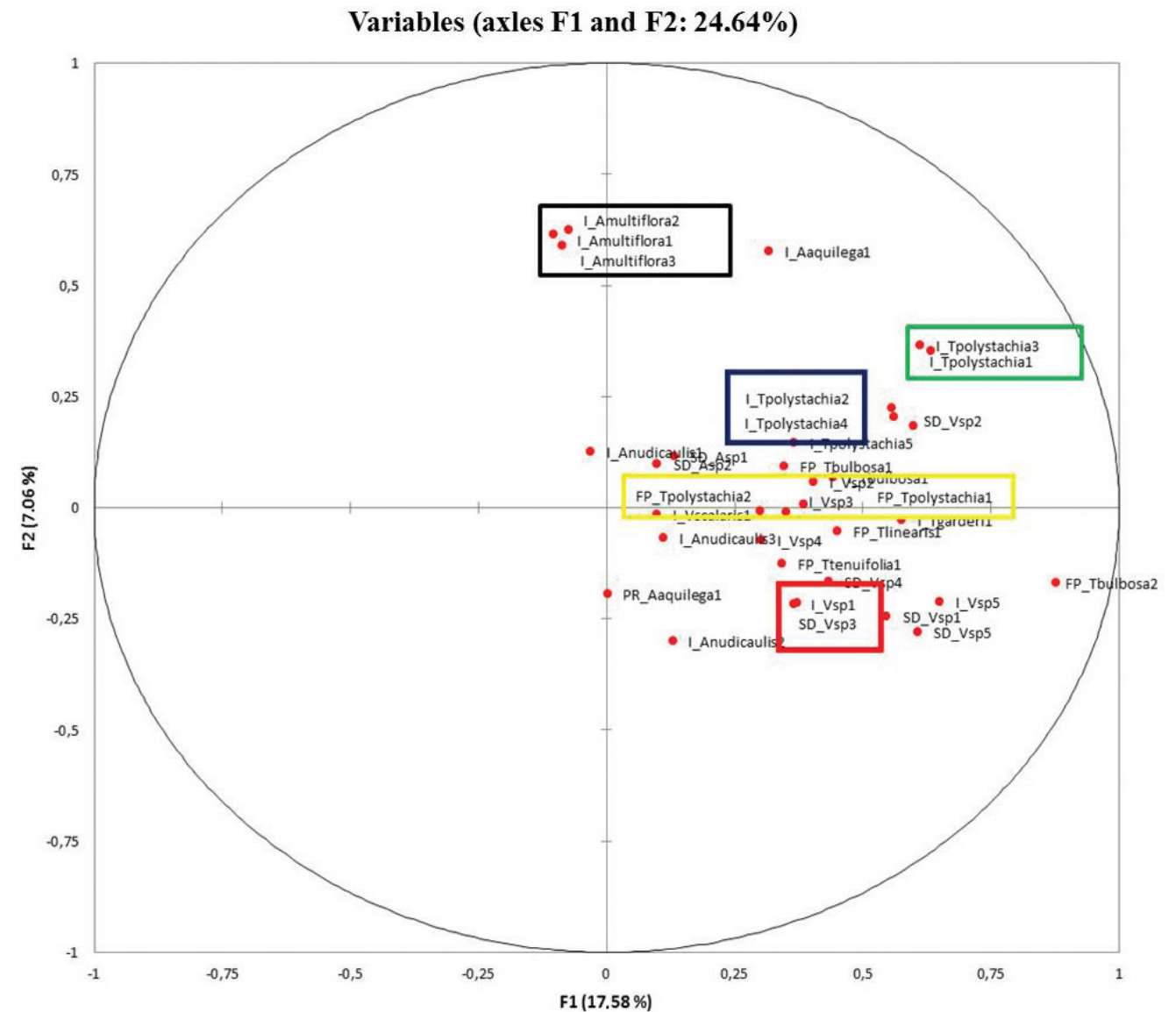


Figure 2. Principal component analysis (PCA) of 34 accessions of the Active Germplasm Bank of bromeliads, through morphological and ISSR markers.

4 Conclusions

The prospection showed a great diversity in the bromeliad species of Sergipe State.

The data generated by ISSR and morphological markers enabled the identification and separation of bromeliad accessions of the AGB.

The analyzed indexes showed high genetic diversity among the bromeliad genera.

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