

Genetic Diversity in *Aechmea fulgens* (Bromeliaceae: Bromelioideae) Revealed by Molecular Markers

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1. Introduction

The Brazilian Atlantic Forest biome contains a high level of genetic diversity and endemic species. In the Atlantic Forest of Northeastern Brazil (Figure 1), habitat loss and fragmentation are the major threats to biodiversity. The degree of degradation of these areas is even greater than that observed in other regions of Brazil (Kimmel et al. 2008). These remnants are only 2% of the original forest area and are mostly restricted to private areas. These forest remnants shelter many native bromeliad species that are threatened with extinction.

Theoretically, population changes associated with habitat degradation lead to an erosion of genetic variation and increased interpopulation genetic divergence due to increased random genetic drift, elevated inbreeding, and reduced gene flow, where remnant/population size and isolation fall below critical levels. These changes are also predicted to affect population viability in the short and long term. In the short term, disturbed plant populations are expected to suffer increased disease and pest susceptibility, loss of incompatibility alleles, and fixation of deleterious alleles. In the long term, loss of genetic variation is expected to reduce the ability of populations to respond to changing selection pressures (Lowe et al. 2005).

The objective of this work was to study the genetic diversity and structure of *Aechmea fulgens* populations through SSR and ISSR molecular markers. Three populations from different remnants of Atlantic Rainforest in Pernambuco state, located in the northeastern region of Brazil, were comparing regarding the level of polymorphism and other populational parameters.

1.1 Bromeliad history

Bromeliads are typical plants of the American continent and probably originated in the Andean region. The species spread out throughout the millennia and reached the tropical

forest about 200,000 years ago. In this taxonomic group, 2700 species were described, all native to the American continent except for a single species, *Pitcairnia feliciana*, which is found in West Africa (Benzing 2000).



Fig. 1. General view of an Atlantic Forest remnant in Northeastern Brazil. Igarassu, Pernambuco

Both terrestrial and epiphyte bromeliad are found even in the most desert and arid regions of the world. Bromeliaceae is subdivided into three sub-families: Bromelioideae, Tillandsioideae and Pitcairnioideae. It is estimated that about 40% of the species dwell in Brazil, many of which are endemic and concentrated mainly in the Atlantic Forest biome (Leme and Marigo 1993).

The bromeliads, being mostly epiphytes, depend on the forest conservation. The devastation process of the Atlantic Forest biome is the main cause of the genetic erosion of the bromeliad species. Only 18.06% of this forest is maintained in form of dispersed fragments in its associated ecosystems (Fundação SOS Mata Atlântica 2001).

The dynamics of tropical forest destruction have led to serious alterations in the ecosystems that compose the respective biomes due in particular to the high fragmentation of the habitats and to genetic erosion (Heringer and Montenegro 2000), making the establishment of germplasm conservation programs necessary and urgent.

1.2 *Aechmea fulgens*

The *Aechmea* genus has significant representation in Pernambuco state where there are 22 species distributed in different environments (Forzza et al. 2010). The *Aechmea fulgens* Brongn. species deserves attention as a plant with economic value because of its ornamental

potential. These species belongs to the Bromelioidea subfamily, Bromeliaceae family, and has epiphyte and soil habit and also occurs on rock outcrops. It can be found in the Atlantic Rainforest from Pernambuco, Bahia as far as Rio de Janeiro. In Atlantic Rainforest the bromeliads account for about 30% of the food resources used by hummingbirds and bats (Sazima et al. 1999). In spite of its great importance for tropical forests, little is known about the reproductive biology of the Bromeliaceae family (Balke et al. 2008). Some species of this family have mechanisms that favor cross pollination, but most are self incompatible. These processes are frequently associated to pollination by vertebrates (Fleming et al. 2009).

The ornamental value and lack of commercial production of the species has led to aggression against the native forests, from where great quantities are collected for commercialization on the domestic and foreign markets. Anthropological action and habitat fragmentation are the greatest causes of erosion of the biodiversity of tropical forests (Schaffer and Prochnow 2002).

The preservation of genetic diversity is one of the main objectives of conservation programs and the first step is knowledge of the distribution of this diversity within and among natural populations (Han et al. 2009). The most important characteristics in population genetics include understanding the gene flow and the genetic diversity and structure, because they are considered essential in the formulation of management and conservation strategies.

1.3 Molecular markers

The polymerase chain reaction (PCR) based on molecular markers such as microsatellite (SSR) and inter-simple sequence repeats (ISSR) are being extensively used to study the genetic diversity in a number of plant species at the species as well as cultivar level. To date, no report is available on applications of molecular markers in studies on the genetic diversity in *Aechmea fulgens* populations. In this investigation, a first attempt was made to study the genetic diversity among three populations of *A. fulgens* using SSR and ISSR markers.

The Microsatellites or SSR (simple sequence repeats) and ISSR (inter-simple sequence repeats) marker methodologies have been widely used in studies with plant populations. ISSR involves amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly ISSR of different sizes. ISSRs exhibit the specificity of microsatellite markers, but need no sequence information for primer synthesis enjoying the advantage of random markers. ISSR markers usually show high polymorphism although the level of polymorphism has been shown to vary with the detection method used. SSR markers often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number is ten or greater. SSRs have been the marker of choice in most areas of molecular genetics as they are highly polymorphic even between closely related lines, require low amount of DNA, can be easily automated for high throughput screening, can be exchanged between laboratories, and are highly transferable between populations. SSRs are mostly codominant markers, and are indeed excellent for studies of population genetics and mapping (Semagn et al. 2006).

2. Material and methods

2.1 Species under study

Aechmea fulgens (Figure 2) is a bromeliad native to the Atlantic Rainforest. In addition to its ecological importance, it has economic importance as a plant with ornamental value. The specie was used in this study because of its wide distribution in the Atlantic Rainforest remnants in Pernambuco state and because of its ornamental potential. As certification that the species under study was indeed *Aechmea fulgens*, a specimen from each collection location was identified and placed in the herbarium at the Instituto Agronômico de Pernambuco (IPA 57000.250).

The species and the collection locations were selected following a survey carried out in the herbariums at the Instituto Agronômico de Pernambuco (IPA), the Pernambuco Federal University (UFPE) and the Pernambuco Federal Rural University (UFRPE). The survey considered: species common to the various regions, most recent data collection and importance of the species. The plant material was collected in remnants of the Atlantic Rainforest in three areas located in Pernambuco - region of Northeastern Brazil (Figure 3). Pieces of young leaves were collected from 20 individuals of each population.

2.2 Study areas

2.2.1 Charles Darwin Ecological Refuge (07°49' S - 34°56' W) - located in the region of Igarassu city on the northern coast of Pernambuco state (Brazil), with an area of approximately 60 hectares, whose vegetation is in the process of recomposition.

2.2.2 Tapacurá Ecological Station (08°04' S - 35°13' W) - located in eastern Pernambuco state in the region of São Lourenço da Mata city, with about 700 hectares. The mission of the Tapacurá Ecological Station is to carry out research in the fields of botany, zoology and ecology. Work at the station is aimed at developing habitats for conserving forest resources and fauna in the Brazilian Atlantic Forest.

2.2.3 Mata do Estado (07° 35' S - 35 ° 30' W) - located in the region of São Vicente Férrer city, in north Pernambuco state, with area of approximately 600 hectares . Of the areas studied, this has suffered most from anthropological action.

2.3 Isolation of genomic DNA

Equal amounts (0.2 g dry weight) of leaf tissue from each sample were placed in porcelain mortars refrigerated with liquid nitrogen and ground to a fine powder. Total genomic DNA was extracted according to the CTAB method described by Doyle and Doyle (1990). RNA was removed by RNaseA treatment. DNA samples were spectrophotometrically quantified at 260 nm. The A₂₆₀/280 reading to each DNA ranged from OD 1.8 to 2.0. After quantification, the DNA was diluted with ultra-pure water to a working concentration of 20 nanograms (ng) μL^{-1} for PCR analysis.

2.4 SSR assays

First 12 pairs of primers were selected, developed by Sarthou et al. (2003) and Boneh et al. (2003) for species of the *Pitcairnia*, *Tillandsia* and *Guzmania* genera, to test the clarity and

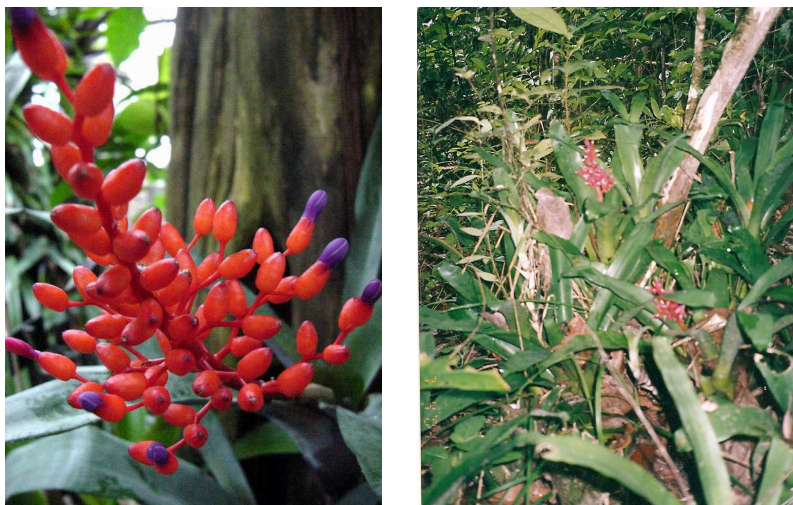


Fig. 2. *Aechmea fulgens*. Population of bromeliads in inner of an Atlantic Forest remnant in Igarassu - Pernambuco - Brazil (on the right) and inflorescence (on the left)

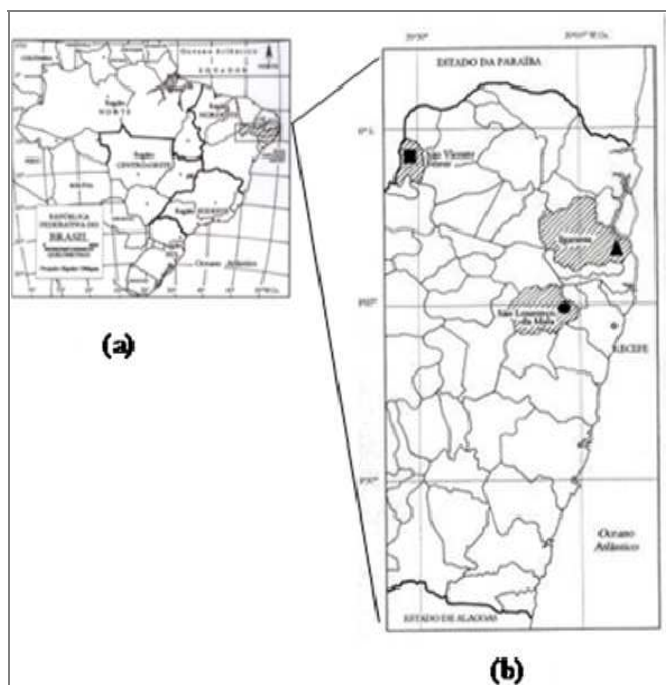


Fig. 3. (a) Pernambuco State - Brazil; (b) Sampling areas for *Aechmea fulgens* populations: ▲ Igarassu, north coast; ● São Lourenço da Mata and ■ São Vicente Férrer, area of forest north.

reproducibility for the analyses. Five pairs of primers were chosen for the amplification reactions that were performed in a TGradient 4.20 Whatman - Biometra (Table 1). With programmed cycles of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C (denaturation); 1 min at 52°C (annealing); and 1 min at 72°C (extension), followed by 7 min at 72°C (final extension of the fragments). Each 20 µL reaction contained 10 mM Tris-HCl (pH 8.0), 2 mM MgCl₂, 100 µM of each triphosphate deoxynucleoside (DNTPs), 0.2 µM of each pair of primers (right and left) from Invitrogen, one unit of the *Taq* DNA polymerase enzyme and 20 ng DNA. The amplification products were resolved in 2.5% agarose gel stained with SyBr Gold (Molecular Probe).

2.5 ISSR assays

The ISSR primers used (Table 2) were selected from a set produced by the University of British Columbia, Vancouver, Canada for *Sphagnum angermanicum* and *Pogonatum dentatum*. The DNA was amplified in a TGradient 4.20 Whatman - Biometra. With the final volume of 25 µL each reaction contained 10 mM Tris-HCl (pH 8.0), 2 mM MgCl₂, 100 µM of each triphosphate deoxynucleoside (DNTPs), 0.2 µM primer from Operon Technologies, one unit of the *Taq* DNA polymerase enzyme and 20 ng DNA. The amplification program was: 94°C for 5 min, 94°C for 30s followed by 30-35 cycles at 50-55 °C, (depending on the primer used). The amplified DNA fragments were separated by electrophoresis in 2.0% agarose gel and stained with SyBr Gold (Molecular Probe).

2.6 Data analysis

2.6.1 ISSR and SSR markers

The ISSR marker fragments visualized in the gels were computed as presence (1) or absence (0). The results of these data generated a binary matrix that was analyzed by the POPGENE Program v. 1.31 (Yeh et al. 1999) to estimate some populational parameters: the percentage of polymorphic loci (*P*); *F* statistics by Wright (Φ_{st}); Nei genetic diversity (*h*) and the Shannon index (*I*). The *F_{st}* values; Φ_{st} were used to estimate the gene flow among the populations $N_m = 1/4 (1/F_{st} - 1)$. The distance between pairs of populations was measured by the Nei genetic distance (Nei 1978). These measurements were then used to construct the dendrogram, using the UPGMA grouping method (Unweighted Pair Group Method with Arithmetic Average). The genetic distance matrix was compared to the geographic distance matrix by the Mantel test to verify whether they were associated. The values of the geographic distance were transformed in \log_{10} for greater proximity to the normal distribution. The Mantel test was carried out by the GENES Program, using 1000 random permutations. The analysis of molecular variance (AMOVA) was used to analyze the distribution of the total within and among genetic variation of the populations, from ISSR marker data using the Program GENES v. 2005.6.1 (Cruz 2001).

The SSR markers were analyzed for the same populational parameters. Because they were co-dominant markers, the data entry, in the genes program, was computed with zero (for absence of fragments), one (for heterozygosity) and 2 (for homozygosity).

3. Results

The primers used to amplify the microsatellite loci (Table 1) generated, depending on the primer, from one to five fragments per locus with sizes ranging from 110 to 450-base pairs

(bp). The transferability of the molecular markers was validated among the *Guzmania* and *Pitcairnia* and *Aechmea* genera by amplifying loci of the same size in species of the referred genera of bromeliad. The percentages of polymorphic loci ranged, respectively, from 80% to 90.3% for the SSR and ISSR markers and the number of alleles per loci ranged from 1.6 to 1.8 for the three populations (Igarassu, São Lourenço da Mata and São Vicente Férrer) and for the two molecular markers used. Figures 3 and 4 represent an example of the polymorphism obtained with SSR and ISSR primers, respectively.

Primer	Derived specie	Sequence (5' - 3')	Size (base pairs)
PgI	<i>Pitcairnia geyskesii</i>	F:TTGAGCCATGAACAATAGGG R:AGAATTCTAGTGGCAGTCCTC	450- 350
PgII	<i>Pitcairnia geyskesii</i>	F:GAGGATGAAGGATTTCCAAGG R:ACCGTCCCACGATAAGAGC	300
PgIII	<i>Pitcairnia geyskesii</i>	F:AACCATTACATGCACCCTCAC R:TCACTGGGGAAGCCATAGAG	200 -100
TGI	<i>Tillandsia fasciculata e</i> <i>Guzmania</i> <i>monostachya</i>	F:CGTACGAAGGTAAGCACAA R:CCGTTGAAGAGGTTAGAGG	250- 110
TGII	<i>Tillandsia fasciculata e</i> <i>Guzmania</i> <i>monostachya</i>	F:AATGAGTTTCAGTTTTAGAAGC R:CCAAGAAAAGAACGGATCA	250 -110

Table 1. SSR primers, derived species, respective base sequences and amplified fragment size in samples of *Aechmea fulgens*.

Table 2 shows the polymorphism and size of the fragments generated by the ISSR markers and reports the ISSR primers that were used and the percentage of polymorphism produced for the set of populations. The number of fragments ranged from eight to 24 and the fragment sizes ranged from 350-2000 bp. The total molecular genetic diversity for each population was observed both by the percentage of polymorphic loci (*P*) and the Shannon-Wiener (*I*) diversity index and the Nei genetic diversity (*h*).

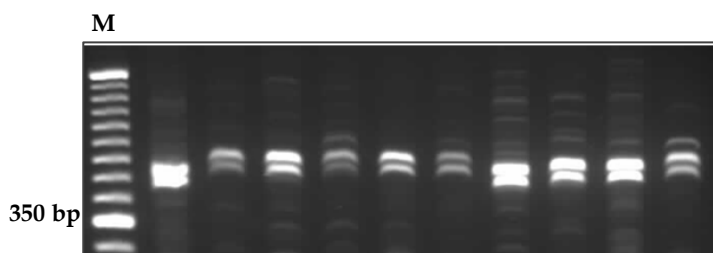


Fig. 3. SSR marker profiles of 10 samples of *A. fulgens* generated by primer PgI in 2.0% agarose gel. M - 50 bp DNA ladder

Primers UBC	Sequence (5' → 3')	Number of fragments	Number of polymorphics fragments	Polymorphism (%)
808	(AG) ₈ C	18	17	94
810	(GA) ₈ T	17	15	90
813	(CT) ₈ T	12	10	88
834	(AG) ₈ YT	12	10	88
842	(GA) ₈ YG	10	10	100
845	(CT) ₈ RG	9	9	100
848	(CA) ₈ RG	7	6	88
890	VHV(GT) ₇	8	7	88
Total		93	84	90,3

The AMOVA analysis, based on 60 ISSR polymorphic loci, showed that 9.1% of the genetic variability was among population and 90.9% within population and the Φ_{st} value was, therefore, equal to 0.0903. The F_{st} (0.040) value detected for the data based on the SSR markers also suggested no differentiation among populations. The estimated gene flow was 2.5 for the analyses with ISSR markers and 5.74 for SSR loci.

Table 2. ISSR primers used and their respective base sequences, number of amplified fragments and polymorphism produced in samples of *Aechmea fulgens*.

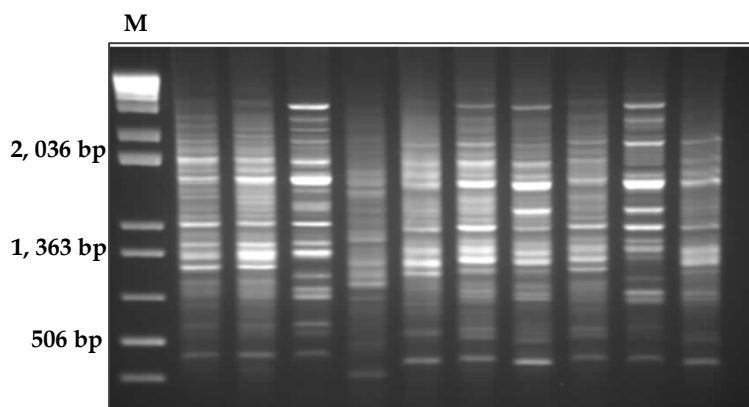


Fig. 4. ISSR marker profiles of 10 samples of *A. fulgens* generated by primer UCB 842 in 2.0% agarose gel. M - 1 kb Plus DNA ladder.

4. Discussion

4.1 Genetic diversity

Many studies of plants confirm the influence of plants' mating mode on the population genetic structure. Populations of clonal plants are expected to be dominated by one or a few locally adapted genets as distinct from plant population with predominant sexual propagation (Jogaité et al. 2005). According to Murawski & Hamrick (1990), the mean

proportion of polymorphic loci for monocotyledons is 40.3%. These authors examined 18 isoenzyme loci in *Aechmea fulgens* populations and detected 21.1% mean polymorphism. The ISSR markers used in this study therefore generated much higher values than those reported by Murawski & Hamrick (1990) for monocotyledons. The same primers used in this study generated similar results in *Grevillea* (Proteaceae) (Pharmawati *et al.*, 2004) and *Cycas guizhouensis* (Cycadaceae) (Xiao *et al.* 2004). In these studies the number of fragments ranged from 8-23 (UCB 808), 7-10 (UCB 842) and 8-24 (UCB 890). Pharmawati *et al.* (2004) obtained fragments for the oligonucleotides UCB 808 (359-1558 bp) and UCB 890 (369-1385 bp), with sizes close to those detected in this study, that ranged from 350-2000 bp. The percentage of polymorphism (90.3%) was close to that reported by Cavallari *et al.* (2006), 89.8%, who studied populations of *Ecolirium biflorum* (Bromeliaceae) with RAPD markers.

Microsatellite molecular markers, widely used in plants, are considered to be very reliable because they are highly specific, usually producing a single fragment, frequently polymorphic at the infrageneric level (Oliveira *et al.* 2006). However, the number of fragments detected depends on the size of the genome, the frequency of the sequences repeated in the genome and the detection method (Barth *et al.* 2002). In the present study, the primers used to amplify the microsatellite loci (Table 1) generated one to five fragments per loci, depending on the primers. The allele pattern produced by the five pairs of primers used in this study permitted the characterization of the *Aechmea fulgens* species with microsatellites developed for use in studies of other species belonging to different genera.

According to Zane *et al.* (2003), microsatellites can be transferred among close species, or even among close genera. The transferability of these markers among related species is a consequence of the homology of the regions flanked by the microsatellites and the size of the region between the pair of primers responsible for amplification by PCR. The possibility of using microsatellites developed for one species in the genetic assessment of another is very useful because it reduces costs considerably. Boneh *et al.* (2003) suggested that the microsatellites they developed for the *Tillandsia* and *Guzmania* genera could be applied to other species within the genus and even to close genera. According to the phylogenetic genetic analysis of the bromeliad family reported by Terry *et al.* (1997), the *Aechmea fulgens* genus is a very different from those referred to by Boneh *et al.* (2003) but two primers used by the these authors were useful in the characterization of *Aechmea fulgens*.

The percentage of polymorphic loci (P) detected in this study was high compared with tropical tree species, for the three populations studied. Auler *et al.* (2002) reported a mean of 43% of polymorphic loci and 1.8 alleles per loci in *Araucaria angustifolia* populations with isoenzymatic markers, and considered the values to be high because these populations had a good conservation 'status' compared to other more degraded populations (20% and 1.4).

The total molecular genetic diversity for each population can be observed both by the percentage of polymorphic loci (P) or by diversity indexes of Shannon-Wiener (I) and Nei's genetic diversity (h).

The values detected for the Shannon-Wiener diversity index in this study were, respectively, for the SSR and ISSR markers: Igarassu: $I = 0.55$ and 0.53 ; São L. da Mata: $I = 0.51$ and 0.51 ; São V. Ferrer: $I = 0.51$ and 0.56 . The Nei genetic diversity ranged from 0.35 to 0.39 in the three populations and for both markers used. Measures of allelic richness are suitable for

assessing short-term diversity loss, but for longer-term loss, involving commoner alleles, measures of gene diversity are more appropriate (Lowe et al. 2004).

4.2 Genetic distances

The Nei genetic distances, calculated for the pairs between populations, ranged from approximately 2% to 7% for the SSR and 4% to 7% for the ISSR markers (Tables 3 e 4). The dendrograms among populations distances for both the molecular markers formed two distinct groups: populations 1 and 3 and population 2 (Figure 5 A and B), where the geographic closer populations were in different groups, suggesting absence of association between geographic distance and genetic distance. This result was confirmed by the Mantel test that showed absence of correlation between the Nei genetic distance matrix and geographic distance for the two markers used. It is important point out that the two types of molecular markers gave the same grouping in spite of their different natures.

As the Nei distance value was proportional to the time since divergence and to the genetic substitution rate by loci and generation (Dias 1998) the values of the genetic distances detected in this study and the grouping formed indicated that the populations did not diverge a long time ago suggesting an explanation for the high within population values and lower among population values.

4.3 Genetic structure

The total genetic diversity revealed that the greatest part of the variation was present within the populations. The results of high within population diversity were in line with several studies with natural plant populations (Auler et al., 2002; Zucchi et al., 2003; Souza et al., 2004; Xiao et al., 2004; Galeuchet et al., 2005; Cavallari et al., 2006). Galeuchet et al. (2005) used seven microsatellite loci and detected $F_{st} = 0.02$ in *Lychmis flos-cuculi* L. (Caryophyllaceae). The authors suggested that the low differentiation among populations studied was primarily due to the significant level of historic gene flow (Nm) when the populations were more connected and second by the contemporary gene flow and accidental anthropogenic seed dispersion.

The Nm value determines whether the gene flow alone can produce substantial genetic variability among locations. If Nm is greater than 1.0, the gene flow will be high enough to prevent differentiation due to drift (Morales & Derbyshire, 2002). In the present study, the estimated gene flow among the populations was high (2.5 and 5.7 for the ISSR and SSR markers, respectively) that together with the high within population genetic variation suggested low impact from the habitat fragmentation on the genetic diversity of *Aechmea fulgens* in the three environments studied. The Nm values, according to Galeuchet et al. (2005), should reflect the historic gene flow, because although bromeliads are probably pollinated by birds and/or bats that have a great flight range, most present clonal propagation. Furthermore, the genetic distance dendrograms (Figure 5 A and B) indicated no divergence among the populations studied.

Foré et al. (1992) studied the genetic structure after fragmentation in 15 *Acer saccharum* (Aceraceae) populations and reported the isolation of remnants with levels of genetic diversity. The authors observed no divergence among the remnants ($F_{st} = 0.03$) and that the gene flow was greater after fragmentation, in this case, due to the greater incidence of wind

and that fragmentation did not result in the isolation of the populations. Souza et al. (2004), however, disagreed with Foré et al. (1992) because they observed high genetic divergence within fragmented populations of *Chorisia speciosa* St. Hil. (Bombacaceae) that indicated, according to the authors, that fragmentation led to genetic drift and to the increase in within population divergence. On the other hand, Nei (1978) stated that when there is reduction in the population size, even though the number of alleles is reduced, the degree of heterozygosity and the genetic diversity can remain as high as in the original population. This is due in most cases to the fact that the alleles lost by drift are rare and contribute little to the level of heterozygosity.

Population (place)	Igarassu	São L. da Mata	São V. Ferrer
Igarassu	-	0.03	0.02
São L. da Mata	9 km	-	0.07
São V. Ferrer	74 km	98 km	-

Table 3. Nei's genetic distance matrix (higher diagonal) and geographical distance (lower diagonal) between pairs of populations of *A. fulgens*. SSR marker data.

Population (place)	Igarassu	São L. da Mata	São V. Ferrer
Igarassu	-	0.06	0.04
São L. da Mata	9 km	-	0.07
São V. Ferrer	74 km	98 km	-

Table 4. Nei's genetic distance matrix (higher diagonal) and geographical distance (lower diagonal) between pairs of populations of *A. fulgens*. ISSR marker data.

The genetic structure of *A. fulgens* populations indicates that *in situ* conservation strategies for this species should be based on the establishment of several small-scale protected areas chosen so as to represent the species' genetic variability. A similar *in situ* conservation schedule was previously suggested by Laguna et al. (1998) for endemic, rare or threatened plant species. Moreover, considering that the samples collected in this study provide a snapshot of the species' distribution area as a whole in Northeastern Brazil and that the genetic variability detected is low, *ex situ* conservation should also be considered in conservation strategies if *A. fulgens* germplasm represents its overall genetic variability.

5. Conclusions

Nowadays, the fragmentation and isolation of natural habitats is one of the main threats to the persistence of many animal and plant species. In this study, we used molecular tools to investigate for the first time the genetic background of the *Aechmea fulgens* populations gene pool. The genetic among population differentiation was small suggesting that the historic gene flow among the populations was still high or that fragmentation did not happen long enough ago to result in pronounced differentiation. Therefore drastic genetic consequences were not shown on the *A. fulgens* populations in the three forest remnants studied.

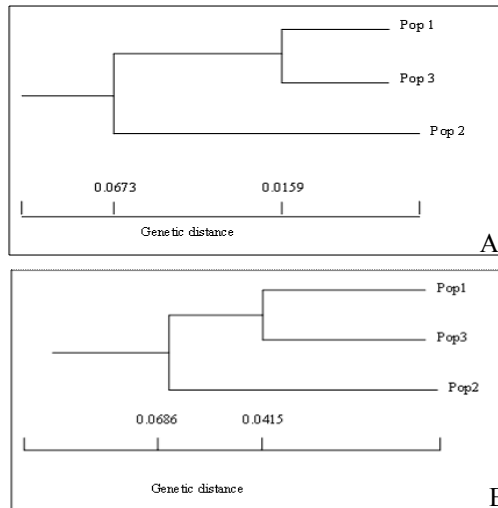


Fig. 5. Nei (1978) Genetic distance dendrogram among the three *Aechmea fulgens* populations. Pop 1: Igarassu; Pop 2: São L. da Mata; Pop 3: São V. Ferrer; A - SSR markers; B - ISSR markers.

Moreover, considering that the samples collected in this study provide a snapshot of the species' distribution area as a whole in Northeastern Brazil and that the genetic differentiation detected is low, *ex situ* conservation should be considered in conservation strategies these bromeliads. Despite the possibility of *ex situ* conservation, *in situ* conservation must be considered as a priority. More extensive studies must be carried out to delineate areas protected by law that effectively help to preserve these species. Furthermore, the potential of the ISSR and SSR markers in studies of population structuring in *A. fulgens* was also demonstrated, this possibly constituting a useful aid in the studies of other bromeliads species.

6. Acknowledgments

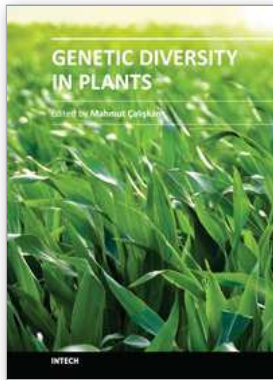
The authors thank the Empresa Instituto Agrônomo de Pernambuco (IPA), Pernambuco, Brazil, for support and infrastructure and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/PADCT), Brazil, for the funding.

7. References

- Auler, N.M.F.; Reis, M.S.; Guerra, M.P. & Nodari, R.O. (2002). The genetics and conservation of *Araucaria angustifolia*: I. Genetic structure and diversity of natural populations by means of non-adaptive variation in the state of Santa Catarina, Brazil. *Genetic and Molecular Biology*, Vol. 25, No. 3, pp. 329-338, ISSN 1415-4757
- Balke, M.; mez-Zurita, J.O.; Ribera, I.; Vilorio, A.; Zillikens, A.; Steiner, J.; García, M.; Hendrich, L. & Vogler, A.P. (2008). Ancient associations of aquatic beetles and tank bromeliads in the Neotropical forest canopy. *Proceedings of the National Academy of Sciences*, Vol. 105, pp. 6356-6361, ISSN 1091-6490
- Barth, S.; Melchinger, A.E. & Lübberstedt T.H. (2002). Genetic diversity in *Arabidopsis thaliana* L. Heynh. investigated by cleaved amplified polymorphic sequence (CAPS)

- and inter simple sequence repeat (ISSR) markers. *Molecular Ecology*, Vol.11, pp.495-505, ISSN 0962-1083
- Benzing, D. H. (2000). *Bromeliaceae: profile of an adaptive radiation*. Cambridge University Press, Cambridge
- Boneh, L.; Kuperus, P. & Van Tienderen, H. (2003). Microsatellites in the bromeliads *Tillandsia fasciculata* and *Guzmania monostachya*. *Molecular Ecology Notes*, Vol. 3, pp.302-303, ISSN 1471-8278
- Cavallari, M.M.; Forzza, R.C.; Veasey, E.A.; Zucchi M.I. & Oliveira, G.C.X. (2006). Genetic variation in three endangered species of *Encholirium* (Bromeliaceae) from Cadeia do Espinhaço, Brazil, detected using RAPD markers. *Biodiversity and Conservation* Vol.15, pp.4357-4373, ISSN 0960-3115
- Cruz, C.D. (2001). *Program GENES: A Software in the Area of Genetics and Experimental Statistics*. Version 2005.6.1 for Windows. Universidade Federal de Viçosa <<http://www.ufv.br/dbg/genes/genes.htm> >
- Dias, L.A.S. (1998). Análises Multidimensionais, In: *Eletroforese de Isoenzimas e Proteínas afins: fundamentos e aplicações em plantas e microrganismos*, Alfenas, A.C. (ed), pp. 201-317, Editora UFV, ISBN 85-7269-027-1, Viçosa, Minas Gerais, Brasil.
- Doyle, J. J & Doyle, J. L (1990). Isolation of plant DNA from fresh tissue. *Focus*, Vol. 12, pp.13-15.
- Fleming, T.H.; Geiselman, C. & Kress, W.J. (2009). The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany*, Vol.104, No.6, pp.1017-1043, ISSN 0305-7364
- Foré, A.S.; Hickey, R.J.; Vankat, J.L.; Guttman, S. & Shaefer R. (1992). Genetic structure after forest fragmentation: a lands ecology perspective on *Acer sacharum*. *Canadian Journal of Botany*, Vol. 70, pp.1659-1668, ISSN 0008-4026
- Forzza, R.C.; Costa, A.; Siqueira Filho, J.A. & Martinelli, G. (March 2010). Bromeliaceae, In: Lista de Espécies da Flora do Brasil, 22.03.2010, Available from <http://floradobrasil.jbrj.gov.br/2010/FB005753/>
- Fundação SOS Mata Atlântica. (2001). SOS Mata Atlântica, <http://www.sosmatatlantica.org.br>, Assessed 09 August 2011
- Galeuchet, D.J.; Perret, C. & Fischer, M. (2005). Microsatellite variation and structure of 28 populations of the common wetland plant, *Lychnis flos-cuculi* L., in a fragmentaed landscape. *Molecular Ecology*, Vol. 14, pp.991-1000, ISSN 0962-1083
- Han, Y.C.; Teng, C.Z.; Wahiti, G.R.; Zhou, M.Q.; Hu, Z.L. & Song, Y.C. (2009). Mating system and genetic diversity in natural populations of *Nelumbo nucifera* (Nelumbonaceae) detected by ISSR markers. *Plant Systematics and Evolution*, Vol. 277, pp. 13–20, ISSN 0378-2697
- Heringer, H. & Montenegro, M. M. (2000). *Avaliação e ações prioritárias para a conservação da biodiversidade da Mata Atlântica e Campos Sulinos*, MMA/SBF, ISBN 85-87166-09-3, Brasília
- Jogaitê, V.; Naujalis, J. R.; Lazutka, J. R. (2005). Genetic diversity in fragmented population of herb-Paris (*Paris quadrifolia* L., Trilliaceae). *Biologija*, No. 2, pp. 5–12.
- Kimmel, T.; Gottsberger, G. & Piechowski, D. (2008). The History of Fragmentation of the Lowland Atlantic Forest of Pernambuco, Brazil. In: *The Fragments Project*, Rodal, M. J. N.; Lins e Silva, A. C. B. & Gottsberger, G. (eds), 1-4, Bioremediation, Biodiversity and Bioavailability, Vol.2 (Special Issue 1), ISSN 1749-0596
- Laguna, E. (1998). *Flora endémica, rara o amenazada de la Comunidad Valenciana*. Generalitat Valenciana, Conselleria de Medio Ambiente, ISBN 84-482-1879-5, Valencia, España
- Leme, E. M. C. & Marigo, L. C. (1993). *Bromélias na natureza*, Salamandra (1ª. Edition), ISBN-13: 97885585352035, Rio de Janeiro

- Lowe, A. J.; Harris, S. A. & Ashton, P. (2004). *Ecological Genetics: Design, Analysis and Application*, Blackwells, Oxford
- Lowe, A. J.; Boshier, D.; Ward, M.; Bacles, C. F. E. & Navarro, C. (2005). Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity*, Vol. 95, pp. 255-273, ISSN 0018-067X
- Moraes, P. L. R. & Derbyshire, M. T. V. C. (2002). Estrutura genética de populações naturais de *Cryptocarya aschersoniana* mez. (Lauraceae) através de marcadores isoenzimáticos. *Biota Neotropica*, Vol.2, pp. 2-17, ISSN 1676-0603
- Murawski, D. A. & Hamrick, J. L. (1990). Local genetic and clonal structure in the tropical terrestrial bromeliad, *Aechmea magdalenae*. *American Journal of Botany*, Vol.77, pp.1201-1208, ISSN 0002-9122
- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, Vol.89, pp. 583-590, ISSN 0016-6731
- Oliveira, E. J.; Pádua, J. G.; Zucchi, M. I.; Vencovsky, R. & Vieira, M. L. C. (2006). Origin, evolution and genome distribution of microsatellites. *Genetics and Molecular Biology*, Vol.29, pp. 294-307, ISSN 1415-4757
- Pharmawati, M.; Yan, G. & McFarlane, I. J. (2004). Application of RAPD and ISSR markers to analyse molecular relationships in *Grevillea* (Proteaceae). *Australian Systematic Botany*, Vol.17, pp. 49-61, ISSN 1030-1887
- Sarthou, C.; Boisselier-Dubayle, M. C.; Lambourdière, J. & Samadi, S. (2003). Polymorphic microsatellites for the study of fragmented populations of *Pitcairnia geyskesii* L. B. a specific saxicolous species of inselbergs in French Guiana. *Molecular Ecology Notes*, Vol.3, pp. 221-223, ISSN 1471-8278
- Sazima, M.; Buzato, S. & Sazima, I. (1999). Bat-pollinated flower assemblages and bat visitors at two Atlantic Forest Sites in Brazil. *Annals of Botany*, Vol.83, pp. 705-712.
- Schaffer, W.B. & Prochnow, M. (2002). *A Mata Atlântica e você*, Apremavi, ISBN 978-85-88733-05-3, Brasília, Brasil.
- Souza, L. M. F. I.; Kageyama, P. Y. & Sebbenn, A. M. (2004). Genetic structure in fragmented populations of *Chorisia speciosa* St. Hil (Bombacaceae). *Scientia Forestalis*, Vol.65, pp. 70-79, ISSN 1413-9324
- Semagn, K.; Bjørnstad, Å. & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, Vol. 5, No. 25, pp. 2540-2568, ISSN 1684-5315
- Terry, R. G.; Brown, G. K. & Olmstead, R.G. (1997). Examination of subfamilial phylogeny in Bromeliaceae using comparative sequencing of the plastid locus *ndhF*. *American Journal of Botany*, Vol.84, pp. 664-670, ISSN 0002-9122
- Xiao, L. Q.; Ge, X. J.; Gong, X.; Hao, G.; Zheng, S. X. (2004). ISSR Variation in the Endemic and Endangered Plant *Cycas guizhouensis* (Cycadaceae). *Annals of Botany (London)*, Vol.94, pp.133-138, ISSN 0305-7364
- Yeh, F. C.; Boyle, T.; Yang, R. C. (1999). POPGENE, the user friendly shareware for population genetic analysis, version 1.31. University of Alberta and Centre for International Forest Research.
- Zucchi, M. I.; Brondani, R. P. V.; Pinheiro, J. B.; Chaves, L. J.; Coelho, A. S. G. & Vencovsky, R. (2003). Genetic structure and gene flow in *Eugenia dysenterica* DC in the Brazilian Cerrado utilizing SSR markers. *Genetics and Molecular Biology*, Vol.26, pp. 449-457, ISSN 1415-4757
- Zane, L.; Bargelloni, L. & Patarnello, T. (2003). Strategies for microsatellite isolation: a review. *Molecular Ecology*, Vol.11, pp.1-16, ISSN 0962-1083



Genetic Diversity in Plants

Edited by Prof. Mahmut Caliskan

ISBN 978-953-51-0185-7

Hard cover, 498 pages

Publisher InTech

Published online 14, March, 2012

Published in print edition March, 2012

Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Clébia Maria Alves de Almeida, Alexandre Gomes da Silva, Maria Tereza dos Santos Correia, Luciane Vilela Resende and Márcia Vanusa da Silva (2012). Genetic Diversity in *Aechmea fulgens* (Bromeliaceae: Bromelioideae) Revealed by Molecular Markers, Genetic Diversity in Plants, Prof. Mahmut Caliskan (Ed.), ISBN: 978-953-51-0185-7, InTech, Available from: <http://www.intechopen.com/books/genetic-diversity-in-plants/genetic-diversity-in-aechmea-fulgens-bromeliaceae-bromelioideae-revealed-by-molecular-markers>

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