Genetic Diversity and Structure of Syagrus romanzoffiana (Cham.) Glassman (Arecaceae) in Southern Brazil

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Abstract
The species Syagrus romanzoffiana is a monocot belonging to the family Arecaceae; it is a palm endemic to South America and is widely distributed throughout the southeast and south of Brazil, including the State of Rio Grande do Sul. To estimate the genetic diversity and structure of the species in the watersheds of Rio Grande do Sul, five simple sequence repeat markers were used to analyze 90 individuals, representing three populations. The genetic structure of S. romanzoffiana groups was evaluated through the analysis of molecular variance and a multivariate analysis based on Nei’s genetic distance. The evaluated populations showed significant intrapopulation molecular variation ($F_{ST} = 0.11$). The observed heterozygosity ($H_O = 0.17$) was lower than the expected heterozygosity ($H_E = 0.75$). These data indicate that although the number of individuals is high, the diversity is low for some indices. Our findings suggest that further studies are needed, particularly on the genetics of natural populations of S. romanzoffiana in Rio Grande do Sul State, to fully characterize their genetic diversity and structure and determine strategies and priority areas for species conservation.

Keywords
molecular ecology, molecular markers, SSR, population genetics, palm, queen palm, pindo

Introduction
The family Arecaceae is listed as having 2,700 species globally, distributed into 240 genera (Dransfield et al., 2008; Lorenzi, Larry, Noblick, Kahn, & Ferreira, 2010). Predominantly present in the tropics, 270 species belonging to 38 genera can be found in Brazil (Lorenzi et al., 2010); this diversity has made palm trees symbols of tropical forests by endemism, wealth, or abundance (Galetti, Pizo, & Morellato, 2003; Jones & Dransfield, 1995).

The species Syagrus romanzoffiana (Cham.) Glassman (Figure 1), popularly known as Coqueiro or Jerivá, is an important species of the Brazilian flora (Lorenzi et al., 2010); this diversity has made palm trees symbols of tropical forests by endemism, wealth, or abundance (Galetti, Pizo, & Morellato, 2003; Jones & Dransfield, 1995).

The species Syagrus romanzoffiana (Cham.) Glassman (Figure 1), popularly known as Coqueiro or Jerivá, is an important species of the Brazilian flora (Lorenzi et al., 2010). Endemic to South America, it is found in Brazil from the south of Bahia to southern Rio Grande do Sul (Noblick, 2017; Reitz, 1974). Present even in archaeological records, this palm tree is widely used by humans since their arrival on the continent as a source of food and fermented beverages, and in dwellings, handicrafts, pigments, fuel, and traditional medicines (Bonomo & Capeletti, 2014; Zambrana et al., 2007). In addition, it is one of the most used ornamental plants in urban landscaping in southern and southeastern Brazil (Lorenzi et al., 2010; Reitz, 1974).

Syagrus romanzoffiana is characterized by the presence of a single stipe, with a diameter ranging from 14 to 45 cm and a height of up to 25 m. Leaves are pinnate, with leaflets up to 25 cm long arranged irregularly on the rachis, giving them a feathery appearance.
Inflorescence is an interfoliate panicle of spiky measuring between 80 and 120 cm that produces light-yellow pistillate or staminate flowers. Blossom and fruiting in this species extend predominantly from October to March, and its fruits are globose-oval drupe with fibrous flesh and an orange-yellow color. The species exhibits remarkable resistance to cold, enduring temperatures of up to $-9^\circ C$ which allows for its distribution in temperate climates. It is also considered hydrophilic owing to its high resistance to extremely wet environments (Curcio, De Sousa, Bonnet, & Barddal, 2007; Glassman, 1972; Goudel, Shibata, Coelho, & Miller, 2013; Lorenzi et al., 2010; Rambo, 1958; Reis, 2006; Reitz, 1974; Soares, Longhi, Witeck Neto, & Assis, 2014; Sobral, 2006).

*S. romanzoffiana* has a fundamental ecological role as an important source of food for several species of mammals, birds, and insects, mainly related to its nutritional characteristics and wide spectrum of fruiting. In addition, this species constitutes an important component of natural ecosystems through interactions with its dispersers and pollinators (Carvalho, 2006; Galetti et al., 2013; Souza et al., 1993; Siqueira, 1989; Terborgh, 1986; Zona & Henderson, 1989).

*S. romanzoffiana* is present throughout the State of Rio Grande do Sul, appearing in different forest habitats, both in the Pampa and Atlantic Forest biomes, predominantly along watercourses (Soares et al., 2014). The presence of this palm tree in areas of the periodic flooding was highlighted by Rambo (1958), and Leite and Klein (1990).

Clarifying genetic diversity of a species is essential to understanding its ecological characteristics such as the delimitations of groupings and the composition of populations. These factors are fundamental to the establishment of conservation strategies and determination of high-diversity priority areas for conservation (Torggler, 1995; Vinson, Kanashiro, Harris, & Boshier, 2015; Yeeh, Kang, & Chung, 1996).

In general, microsatellite markers provide essential information for estimating genetic parameters of interest in population genetics (Ferreira & Grattapaglia, 1998; Gupta et al., 2003); these are also known as simple sequence repeats (SSRs), which are short DNA sequences of 1 to 6 base pairs repeated in tandem that can be detected by polymerase chain reaction (PCR) using specific primers (Litt & Luty, 1989; Stefenon, 2010; J. L. Weber & May, 1989; Zucchi et al., 2003). Allelic and genotypic information obtained from these markers is useful for understanding the genetic diversity, genetic structure, and phylogeographic patterns of natural populations (e.g., Stefenon, Gailing, & Finkeldey, 2007), aiding in formulating conservation strategies for genetic resources (Gao, Schaal, Zhang, Jia, & Dong, 2002; Powell, Machray, & Provan, 1996; Victoria, Da Maia, & De Oliveira, 2011).

On the basis of the characteristics related to the occurrence of a species within an environment and the molecular analysis tools currently available, ecological studies on the relationship between a watershed and the molecular variability of a given species may be useful for inferring if hydrographic basins are limiting for species distribution. The use of the watershed approach intrinsically simulates...
the space, that is, the patterns and processes occurring within a spatial unit called the watershed (Schiavetti & Camargo, 2002). In the State of Rio Grande do Sul, *S. romanzoffiana* is found within three main watersheds, namely Guaiaba, Litoral, and Uruguai; therefore, it may be a valuable model for testing this hypothesis. On the basis of these premises, this study aimed to evaluate the genetic diversity and structure of three populations of *S. romanzoffiana* in Rio Grande do Sul State, each growing within one of the three watersheds, to determine the influence of the hydrographic basin on the allelic distribution of this species.

**Material and Methods**

**Study Area**

This study was carried out in the State of Rio Grande do Sul in the southernmost region of Brazil. The state covers an area of approximately 281,748 km², with an altitude varying from sea level up to 1,398 m, and a climate classified predominantly as Cfa, with a restricted portion being classified as Cfb (Koppen–Geiger). It comprises two distinct biomes, the Atlantic Forest and Pampa (Kuinchtner & Buriol, 2001; E. Weber et al., 1998).

In this study, the three watersheds dividing the territory of the Rio Grande do Sul State were used as limiting factors for determining the populations of *S. romanzoffiana*. The Litoral watershed consists of the easternmost and southernmost regions of the state, presenting altitudes from 0 to 100 m above sea level (ASL) and covering mainly the geomorphological province of Planicie Costeira. The Guaiaba watershed is located in the central-eastern part of the state, with altitudes ranging between 100 and 800 m ASL and covering the geomorphological regions of the Southern Plateau and Central Depression. The Uruguai watershed covers the extreme north and western regions of the state, with altitudes ranging between 0 and 1,200 m ASL and corresponds to the geomorphological unit of the Southern Plateau.

**Sample DNA Isolation and PCR Essays**

Foliar samples from 90 adult individuals of *S. romanzoffiana* (30 plants each watershed; Figure 2) were obtained on expeditions performed between March and December 2016. All specimens sampled were georeferenced using a Gamin 26 GPS; leaflets were identified with a reference number and then stored in an ultra-freezer at −80°C.

Total DNA was isolated from the sampled leaflets with a Qiagen Plant MiniKit®, following the manufacturers’ instructions. Quantity and quality of the isolated DNA were evaluated in a NanoVue™ Plus spectrophotometer. The PCR conditions were adapted from Geethanjali, Rukmani, Rajakumar, Kadirvel, and Viswanathan (2017). Five molecular markers developed for *Livistona chinensis* (Jack.) R. Br. (Ohtani, Tani, & Yoshimaru, 2009), *Cocos nucifera* L. (Teulat et al., 2000), and *Phoenix dactylifera* L. (Elshibli & Korpelainen, 2008; Table 1) were used for genotyping the 90 samples of *S. romanzoffiana*, using 2 μL of DNA, 1 μL of oligonucleotide initiators, continuous and reverse; 1.5 μL of Buffer (10×); 1 μL MgCl₂ (5 U/μL); deoxyribonucleotide triphosphates 1 μL (100 mM); 0.2 μL Taq Polymerase buffer (5 U/μL), and 7.3 μL of ultrapure water (Milli-Q) for a final volume of 20 μL. Amplification was performed in a BIO-RAD C1000 Touch™ thermocycler, with an initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s for denaturation, 1 min at 51°C for annealing, 1 min extension at 72°C, and a final extension at 72°C for 10 min.

The PCR products were resolved on a 3% agarose gel at 80 V, stained with GelRed™ (Biotium®) and visualized under ultraviolet light. A 100-base pair marker ladder (Norgen) was used for sizing the alleles. Images were generated with the LPix EX-2.6-PGR program (Locus Biotecnologia) and fragment size was determined using the TotalLab TL120 gel analysis program.

**Data Analysis**

Total number of alleles (*A*), effective number of alleles (*Ae*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and inbreeding coefficient, *F* = (*He* − *Ho*)/*He*, were estimated for each locus and multiloci for each population. The genetic structure of *S. romanzoffiana* populations were evaluated through the analysis of molecular variance (AMOVA; Excoffier, 1993) and a multivariate analysis (PCoA) based on Nei’s (1978) genetic distance. All analysis were performed using the GenAlEx 6.4 software (Peakall & Smouse, 2012).

**Results**

**Levels of Genetic Diversity**

Overall, the five loci evaluated in the 90 individuals of *S. romanzoffiana* were polymorphic and generated 82 alleles. Locus MSr27 comprised the highest number of alleles (22), while the smallest number (14 alleles) was observed for the MSr01 and MSr02 loci.

At the population level, all loci were polymorphic in the Guaiaba and Uruguai populations, whereas locus MSr02 was monomorphic and presented an elevated level of missing data in the Litoral population. The number of alleles per population ranged from 40 in the Guaiaba population to 28 in the Litoral population.
Private alleles were found in all populations: five in Guaíba, three in Litoral, and three in Uruguai. Multilocus estimations of observed heterozygosity ranged from $H_O = 0.10$ (Guaíba population) to $H_O = 0.28$ (Uruguai population), while estimations of expected heterozygosity ranged from $H_E = 0.59$ (Litoral population) to $H_E = 0.83$ (Guaíba population). Consequently, estimations of fixation index were
relatively high, ranging from $F = 0.65$ (Uruguai population) to $F = 0.88$ (Guaiba population), indicating a high level of inbreeding (Table 2).

**Population Genetic Structure**

The AMOVA based on the five polymorphic loci revealed that 11% of the observed genetic variability was distributed among populations, 82% among individuals within the populations, and 7% among individuals (Table 3). Estimations of $F$ statistics (Weir & Cockerham, 1984) were high and statistically significant ($p < .001$) among populations ($F_{ST} = 0.110$), within populations ($F_{IS} = 0.925$), and overall ($F_{IT} = 0.933$).

The estimated gene flow based on pairwise $F_{ST}$ ranged from $Nm = 1.73$ to $Nm = 2.34$ migrants per generation. As expected, these values are directly correlated to the estimations of Nei’s genetic distance (Table 4).

**Table 1. SSR Markers Used in This Study.**

| Marker   | Sequence                  | Product size (pb) |
|----------|---------------------------|-------------------|
| MSr01a   | F:AACGTGAAAGACGATT        | 206–290           |
|          | R:ACGAGGAGGATCAAGACGATT   |                   |
| MSr02a   | F:CATGGAATTGTAATGGAGGAGG | 170–230           |
|          | R:TATCTTCTCTCTCTCTCTCTCT |                   |
| MSr03a   | F:ACGGCAATGGAGGATCAAGAC  | 240–296           |
|          | R:CGTCTCTCTCTCTCTCTCTCT |                   |
| MSr27b   | F:AGCAAGGTCGCGAAGAAGA    | 220–300           |
|          | R:ACGAGGAGGATCAAGACGATT  |                   |
| MSr28b   | F:ATTCATTATTCAGACCAAC    | 78–124            |
|          | R:GGTCTCTCTCTCTCTCTCTCT |                   |

**Note.** SSR = simple sequence repeat.

*Livistona chinensis* (Jack) B. Br.

*Cocos nucifera* L.

*Phoenix dactylifera* L.

**Table 2. Genetic Diversity Parameters Estimated for Syagrus romanzoffiana in the Rio Grande do Sul watersheds.**

| Population | %Polym. | Locus | $A$ | $Ae$ | $H_{O}$ | $H_{E}$ | $F$  |
|------------|---------|-------|-----|------|---------|---------|------|
| Guaiba     | 100%    | MSr01 | 8.00| 4.17 | 0.00    | 0.77    | 1.00 |
|            |         | MSr02 | 6.00| 3.92 | 0.10    | 0.78    | 0.87 |
|            |         | MSr03 | 12.0| 8.23 | 0.42    | 0.92    | 0.53 |
|            |         | MSr27 | 8.00| 5.92 | 0.00    | 0.84    | 1.00 |
|            |         | MSr28 | 8.00| 5.70 | 0.00    | 0.82    | 1.00 |
|            |         | Multiloci | 8.40 | 5.59 | 0.10   | 0.83    | 0.88 |
| Litoral    | 80%     | MSr01 | 6.00| 4.91 | 0.00    | 0.82    | 1.00 |
|            |         | MSr02 | 1.00| 1.00 | 0.00    | 0.00    | –    |
|            |         | MSr03 | 6.00| 3.27 | 0.50    | 0.76    | 0.28 |
|            |         | MSr27 | 9.00| 3.73 | 0.03    | 0.75    | 0.95 |
|            |         | MSr28 | 6.00| 2.74 | 0.14    | 0.75    | 0.78 |
|            |         | Multiloci | 5.60 | 3.13 | 0.13   | 0.59    | 0.75 |
| Uruguai    | 100%    | MSr01 | 9.00| 6.25 | 0.00    | 0.68    | 1.00 |
|            |         | MSr02 | 8.00| 8.00 | 1.00    | 1.00    | –0.14|
|            |         | MSr03 | 5.00| 2.98 | 0.40    | 0.70    | 0.40 |
|            |         | MSr27 | 9.00| 6.54 | 0.00    | 0.86    | 1.00 |
|            |         | MSr28 | 6.00| 3.08 | 0.00    | 0.69    | 1.00 |
|            |         | Multiloci | 7.40 | 5.37 | 0.28   | 0.82    | 0.65 |

%Polym. = percentage of polymorphism per population; $A$ = number of alleles per locus; $Ae$ = effective number of alleles; $H_{O}$ = observed heterozygosity; $H_{E}$ = expected heterozygosity; $F$ = fixation index.
the 90 genotyped individuals, only 6 presented tendencies to belong to a genetic population different from that of origin (Table 5).

**Discussion**

There are several ways to explain the current distribution pattern of a species. Although *S. romanzoffiana* is not considered a threatened species, the populations evaluated in this study presented moderate levels of allelic diversity, but markedly low levels of $H_O$. Habitat fragmentation and opening of space for agriculture and urbanization, with consequent loss of dispersers and pollinators in the Pampa and Atlantic Forest biomes, threatens the viability of the species mainly because of loss of genetic diversity (Murcia, 1995; Roesch et al., 2009; Wright & Duber, 2001; Wright et al., 2000).

The low levels of $H_O$ estimated in this study may be an effect of the transferability of the employed SSR loci. Significantly lower estimations of $H_O$ (0.148) in relation to $H_E$ (0.695) were observed in *Acromia emensis* (Tol.) Lorenzi (Arecaceae) in an analysis based on SSR markers transferred from other species (Neiva et al., 2016). In addition, lower observed heterozygosity ($H_O = 0.164$) was observed in comparison to expected heterozygosity ($H_E = 0.262$) in *Elaeis oleifera* (Kunth) Cortés (Arecaceae) genotyped with species-specific SSR markers (Zaki, Singh, Rosli, & Ismail, 2012).

This low level of $H_O$ is detrimental to a species as a low input from heterozygotes makes populations vulnerable to environmental changes, limiting the ability to respond and adapt (Avise, Bowen, Lamb, Meylan, &

**Table 3.** AMOVA for Populations of *Syagrus romanzoffiana* in Rio Grande do Sul.

| Source      | df   | SS    | MS    | VT% |
|-------------|------|-------|-------|-----|
| Among pops  | 2    | 30.31 | 30.31 | 11  |
| Among indiv | 87   | 271.30| 6.24  | 82  |
| Within indiv| 90   | 11.00 | 0.12  | 7   |
| Total       | 179  | 312.61| 100   |     |

Note. $F_{ST} = 0.110$ ($p < .001$), $F_{IS} = 0.925$ ($p < .001$), $F_{IT} = 0.933$ ($p < .001$). $df = $ degrees of freedom; SS = sum of squares; MS = mean squares; VT% = total variance; $F_{ST}$ = genetic divergence among populations.

**Table 4.** Number of Migrants (Below Diagonal) Between Populations Per Generation, and Nei’s Genetic Distance (Above Diagonal).

|        | Guia | Litoral | Uruguai |
|--------|------|---------|---------|
| Guia   | –    | 1.05    | 1.27    |
| Litoral| 1.73 | –       | 1.09    |
| Uruguai| 2.34 | 1.81    | –       |

Note. Only samples assigned to a population different from the population origin are presented. The numbers in bold represents the genotyped individuals that presented tendencies to belong to a genetic population different from the original and are sampled ($p > 0.01$).

**Table 5.** Assignment Analysis of Individual Samples.

| Sample | Origin population | Litoral | Uruguai | Assigned population |
|--------|-------------------|---------|---------|---------------------|
| 24     | Guia              | -6.64   | -6.63   | 12.00 Litoral       |
| 25     | Guia              | -7.76   | -9.56   | -6.64 Uruguai       |
| 35     | Litoral           | -3.69   | -4.12   | 6.75 Guia           |
| 40     | Litoral           | -6.77   | 7.82    | 10.45 Guia          |
| 67     | Uruguai           | -4.74   | -4.95   | 5.95 Guia           |
| 78     | Uruguai           | -9.20   | -9.56   | 10.72 Guia          |

Note. Only samples assigned to a population different from the population origin are presented.
Excessive inbreeding resulting either from habitat fragmentation or a species characteristic, leads to significant loss of diversity by a decrease or isolation of a population; to redress this balance, a large input of individuals over a long period of time is needed as inclusion of an allele occurs by random crossing (Hamrick, Godt, & Shermam-Broyles, 1992).

Palynological studies in the study area have indicated that S. romanzoffiana emerged approximately 940 radio-carbon years ago and is associated with the emergence of ciliary vegetation. In the same period, the forest diversity of the gallery to the southeast of South America expanded exponentially (Moureelle, Prieto, & Garcia-Rodriguez, 2017).

In a study of communities of palm trees in the western Amazon, Kristiansen et al. (2012) verified that there was a correlation between species distribution and the flood environment. Consequently, it was possible to classify palm trees according to hydrology, in addition to the dispersion environment where geographical distance was the main limiting factor. In addition, events such as flood times and resurgence of land areas (Crisp, Trewick, & Cook, 2011), which determined the distribution pattern of the hydrophilic terrace, may be related to the current distribution pattern of the palm.

Factors contributing to these population dynamics may be explained by relationships with pollinators. According to Nogueira-Neto (2002), pollination of S. romanzoffiana is carried out by Apis mellifera, several species of the tribe Meliponini, and Jatai bees attracted by its flowers containing abundant pollen, in addition to species of Coleoptera that reproduce in the flowers of the palm tree and consequently contribute to dispersal. Moreover, large mammals are also important dispersers as they normally occupy large areas. In this sense, the extent of dispersal may also contribute to the spatial organization of S. romanzoffiana populations (Budke, Athayde, Giehl, Záchia, & Eisinger, 2005; Freire, Closel, Hasui, & Ramos, 2013; Giombini, Bravo, & Martínez, 2009; Messias & De Assis Alves, 2009).

Adjacent area studies by Terral et al. (2012) suggested that routes of human dispersion may have contributed to the geographical distribution pattern of P. dactylifera L. (Arecaceae), as the indigenous populations made daily use of the palm. Human dispersion may also have contributed to the distribution of S. romanzoffiana as indigenous populations in South America traveled long distances along riverbeds and may have transported the seeds; indeed, several archaeological studies have reported palm use by indigenous groups in food, house covering, and traditional medicines (Bonomo & Capeletti, 2014). The ornamental use of this species in urban areas such as in roads, squares, and gardens of southern Brazil (Lorenzi et al., 2010; Reitz, 1974) may also affect its natural organization, although to a lesser extent.

The level of genetic divergence among populations estimated as $F_{ST} = 0.11$ indicates moderate differentiation (Slatkin, 1987). Interpopulation gene flow is important for avoiding significant genetic distinctions within populations because they would be subject to evolutionary pressures, endogamy, and genetic drift (Nagel & Stefanon, 2013; Serrote, Reiniger, & Stefanon, 2016; Slatkin, 1987). Considering that 11% of the observed differentiation occurred among populations, as well as the significant geographical distribution of plants along the three watersheds, we propose that gene flow is efficient among populations. Nevertheless, approximately 84% of the plants had a genetic identity different to their population of origin, resulting from differences in allelic frequencies among populations and the presence of private alleles.

Finally, the results of this study were inconclusive in relation to the influence of the hydrographic basin on the distribution of alleles. A larger number of molecular markers are needed to elucidate this issue. However, our results shed some light with regard to conservation as they provided the first estimate of genetic diversity in the S. romanzoffiana palm. Although the risk of extinction is low, according to the Red List—International Union for Conservation of Nature (Govaerts & Dransfield, 2005), our analysis showed low levels of $H_D$ and extremely high inbreeding in the evaluated populations. This outcome suggests that further studies on the genetic characteristics of natural populations of S. romanzoffiana in Rio Grande do Sul State are needed to fully characterize their genetic diversity and structure, and to determine strategies and priority areas for species conservation.

**Implications for Conservation**

This study suggests that the assessment criteria used to determine the vulnerability this species may not reflect the true state of conservation; a large population may not guarantee the survival of S. romanzoffiana as our results demonstrated the presence of low diversity.

**Declaration of Conflicting Interests**

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