



Transferability of microsatellite markers to *Syagrus harleyi* (Mart.) Becc. (Arecaceae), an endemic species from the Diamantina Plateau, a biodiversity hotspot in northeastern Brazil

Leydiane da Conceição Lazarino¹ , Derval Gomes Pereira¹ , Lorena Souza Silva¹
Renata Miranda Souza¹ , José Henrique Galdino^{1*} , Ana Maria Waldschmidt¹

¹Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia (UESB), 45205-490, Jequié, BA, Brasil. E-mail: galdino.jhsgb@gmail.com. *Corresponding author.

ABSTRACT: *Syagrus harleyi* is a stemless palm endemic to a small area in the Diamantina Plateau (Bahia, northeastern Brazil), a biodiversity hotspot in South America. The natural range of this species has been intensively affected by landscape fragmentation and fire episodes. In turn, no information about the levels of genetic variation and the evolutionary potential of *S. harleyi* is available so far. Thus, this study evaluated the cross-amplification of microsatellite (SSR) primers developed from three phylogenetically related palm species to *S. harleyi*. A total of 30 individuals from three populations were analyzed using 19 heterologous microsatellite loci. Based on 10 polymorphic loci, we identified a mean number of 4.1 alleles per locus and mean values of expected and observed heterozygosity of 0.579 and 0.780, respectively. These results showed that these loci are fully transferable and could be effectively used for genetic diversity assessment of *S. harleyi* populations focused on their management and conservation.

Key words: palm tree, endemic species, caatinga, SSR, cross-amplification.

Transferibilidade de marcadores microssatélites para *Syagrus harleyi* (Mart.) Becc. (Arecaceae), uma espécie endêmica da Chapada Diamantina, um hotspot de biodiversidade no Nordeste do Brasil

RESUMO: *Syagrus harleyi* é uma palmeira acaulescente endêmica da Chapada Diamantina (Bahia, Nordeste do Brasil), um hotspot de biodiversidade na América do Sul. A área de ocorrência da espécie tem sido afetada por incêndios e pela fragmentação da paisagem. Porém, sua diversidade genética permanece desconhecida. Este estudo avaliou a transferibilidade de 19 primers de microssatélites (SSR) desenvolvidos para três espécies filogeneticamente próximas a *S. harleyi*, em 30 indivíduos de três populações. Foram identificados 10 loci polimórficos, com média de 4,1 alelos por locus e heterozigotidade esperada e observada de 0,579 e 0,780, respectivamente. Estes resultados demonstram a eficiência da transferibilidade desses loci o potencial informativo dos mesmos para acessar a diversidade genética em populações de *S. harleyi*, o que contribuirá para o manejo e a conservação dessa espécie.

Palavras-chave: palmeira, espécie endêmica, caatinga, SSR, amplificação cruzada.

The stemless palm *Syagrus harleyi* Glassman is endemic to the Caatinga biome (GONÇALVES & AZEVEDO GONÇALVES, 2023), being restricted to the Diamantina Plateau (northern Espinhaço Range), state of Bahia (Northeastern Brazil), an ecoregion referred to as a major biodiversity hotspot in South America (ASSUNÇÃO-SILVA & ASSIS, 2022). Like most palm trees, *S. harleyi* flowers multiple times a year, representing a crucial species to avoid the collapse of local communities by providing resources for frugivorous and pollinators (BARFOD et al., 2011). Despite its ecological importance, vulnerability, and endemism (FUNCH et al., 2021), much about the

genetic diversity and conservation status of *S. harleyi* remains unknown. In this regard, studies based on molecular markers, such as Simple Sequence Repeats (SSRs), or microsatellites, have been highly useful for the conservation and management of native organisms as they provide reliable estimates of genetic diversity, population structure, gene flow, and adaptive potential (LAZARINO et al., 2023; MOREIRA, 2022).

Conversely, microsatellite analyses often require the development of species-specific primers, which is an expensive process (FASANELLA et al., 2019), particularly for developing and biodiverse countries such as Brazil. However, cross-

amplification using SSR primers developed for closely related species (typically sharing homologies at microsatellite loci) offers a cost-effective alternative for assessing the genetic diversity of understudied groups (SIMPLICIO et al., 2017; FASANELLA et al., 2019). Therefore, considering the vulnerability status and the lack of genetic information on *Syagrus harleyi*, we tested the transferability of heterologous SSR markers and their suitability in estimating genetic parameters in this species.

Leaflet and inflorescence samples were collected from 30 *Syagrus harleyi* specimens (Figure 1), with ten individuals sampled from each of three sites along the Diamantina Plateau: Palmeiras (12°26'35.2"S; 41°29'5.9"W, 701 m.a.s.l.), Andaraí (12°52'5.1"S; 41°18'29.7"W, 522 m.a.s.l.), and Ibicoara (13°24'2.9"S; 41°14'4.7"W, 1075 m.a.s.l.) (Figure 2). Exsiccates of all specimens were identified and deposited in the Herbarium of the State University of Southwestern Bahia (HUESB). Leaflet samples were stored at -20 °C, and afterwards, total DNA was isolated according to ARRUDA et al. (2017).

A set of 19 previously published SSR primer pairs was picked out for assessment of their transferability to *Syagrus harleyi* (Table S1), of which eleven were designed for coconut (*Cocos nucifera*) (PERERA et al., 1999; RIVERA et al., 1999), six for peach palm (*Bactris gasipaes*) (BILLOTTE et al., 2004; RODRIGUES et al., 2004), and two for jussara palm (*Euterpe edulis*) (GAJOTTO et al., 2005) (Table S1). SSR-PCR reactions were performed according to SCHUELKE (2000). All forward primers were

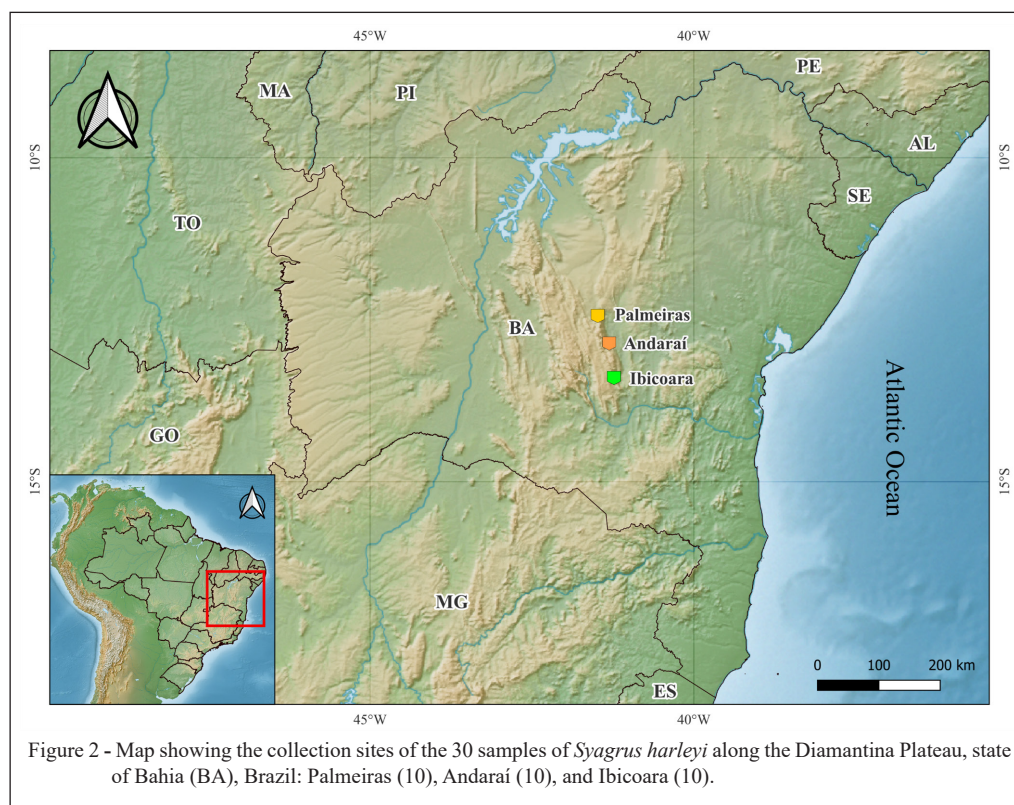
labeled at 5' – ends with 6 – Carboxyfluorescein (6-FAM) fluorescent dye for further genotyping, and gradient PCR was applied to test the amplification efficacy of the cross-genera SSR markers. PCR products were subjected to electrophoresis on 8% polyacrylamide gel to check for amplification and concomitantly select the SSR markers that yielded polymorphic bands.

The selected 6-FAM labeled PCR products were genotyped by capillary electrophoresis on an ABI 3500xL Genetic Analyzer (Applied Biosystems) using the GeneScan™ 500 LIZ™ Size Standard as an internal reference for fragment sizing. Then, the fragment sizes (alleles) were determined with the software Geneious (www.geneious.com). The software Microchecker v. 2.2.0.3 was used to detect the presence of putative null alleles (VAN OOSTERHOUT et al., 2004). Afterwards, the values related to the number of alleles per locus (Na), percentage of polymorphic loci (PLP), expected heterozygosity (He), observed heterozygosity (Ho), inbreeding coefficient (F_{IS}), and deviations from the Hardy-Weinberg equilibrium (HWE) for each marker were estimated in the software GenAlEx (PEAKALL & SMOUSE, 2012).

Out of the 19 SSR primers tested (Table S1), 10 were effectively amplified in DNA samples of *Syagrus harleyi* (Table 1). The optimal annealing temperature for each one is shown in table 1. These primers yielded reproducible polymorphic bands for all populations and were thus selected for the present analyses. A total of 41 alleles were detected, with a mean and range of 4.1 and 3 – 8 alleles/locus,



Figure 1 - The stemless palm *Syagrus harleyi*. A wild plant of *S. harleyi* (A). An inflorescence of *S. harleyi* (B). Fruits of *S. harleyi* (C). The photographs were taken during field expeditions in the Diamantina Plateau.



respectively, with amplicon sizes ranging from 79 to 242 bp (Table 1). The expected heterozygosity (H_e) ranged from 0.514 to 0.644, with an average of 0.579, while the observed heterozygosity (H_o) ranged from 0.523 to 0.926, with a mean value of 0.780. Except for EE54, values of $H_o > H_e$ and negative values of the inbreeding coefficient (F_{IS}) were observed for all microsatellite loci (Table 1), indicating an excess of heterozygosity or absence of inbreeding within populations. Also, most loci (six out of ten) showed significant deviations from the HWE in the tested populations (Table 1).

The successful cross-amplification of 10 SSR loci in *Syagrus harleyi* with high reproducibility across populations confirms the suitability of these markers for the genetic diversity assessment of this palm species. The high transferability rate reported here may be related to the phylogenetic relationship among species. Accordingly, recent phylogenomic studies in the family Arecaceae reinforced that (i) the genera *Syagrus* and *Cocos* are closely related (FRANCISCONI et al., 2023), (ii) the genus *Bactris* is a member of the tribe Cocoseae along with *Syagrus* and *Cocos* (SANTOS DA SILVA et al., 2021), (iii) and the genus *Euterpe* also belongs to the subfamily Arecoideae (CHEN et al., 2022). Furthermore,

successful cross-amplification of SSR markers has been reported in other species of *Syagrus*, such as *S. coronata* (SIMPLICIO et al., 2017), *S. romanzoffiana* (LAINDORF et al., 2019), and *S. oleracea* (SANTOS et al., 2020).

The H_e values (0.514 to 0.644) (Table 1) indicated moderate to high genetic diversity, which is important for the long-term maintenance of populations (SIMPLICIO et al., 2017). Besides, nine loci exhibited values of H_o greater than H_e , and six SSR markers showed significant deviation from HWE (Table 1). A similar pattern was observed in *Syagrus coronata*, another palm species endemic to northeastern Brazil, where up to five SSR loci significantly deviated from Hardy–Weinberg equilibrium (SIMPLICIO et al., 2023). Therefore, despite the moderate to high genetic diversity recorded in our sampled populations, the presence of HWE deviations suggested that they might be susceptible to admixture or evolutionary forces such as genetic drift or local selection. As the purpose of this study was to identify markers transferable to *Syagrus harleyi*, further endeavors should employ a larger sample size for each population that was here analyzed, as well as a broader sampling in the range span of this palm, to a deeper examination of the evolutionary processes shaping their genetic diversity and structure.

Table 1 - Features and genetic diversity statistics for 10 cross-genera SSR loci evaluated in 30 specimens of *Syagrus harleyi*.

Locus	Source species	-----Primer sequence 5'-3'-----	Size range (bp)	T _a (°C)	Na	He	Ho	HWE	F _{IS}
mBgCIR091 ^a	<i>Bactris gasipaes</i>	F:CAAGAACAGGCTCAGTCTA R: TGCAATCAACCCAAGAT	93-167	56	4	0.618	0.635	ns	-0.038
Bg02-09 ^b	<i>Bactris gasipaes</i>	F:CGCAGCAGCAGCAATAAATA R: TCCAGCAACTTTCAGTCGAG	92-166	56	5	0.514	0.921	*	-0.794
Bg02-10 ^b	<i>Bactris gasipaes</i>	F: ATTGGGTCCAGATCCTCTTT R: GTGGCACACATGGGGTTC	187-190	58	3	0.549	0.926	ns	-0.686
mBgCIR066 ^a	<i>Bactris gasipaes</i>	F: GCATGTTGCATTGACTA R: GAATCCTGGTTCAGATACT	93-164	50	4	0.644	0.886	**	-0.497
CNZ10 ^c	<i>Cocos nucifera</i>	F:CCTATTGCACCTAAGCAATTA R: ATGATTTTCGAAGAGAGGTC	93-160	56	3	0.621	0.880	*	-0.451
CNZ51 ^d	<i>Cocos nucifera</i>	F:CTTTAGGGAAAAAGGACTGAG R:ATCCATGAGCTGAGCTTGAAC	160-242	56	8	0.524	0.870	*	-0.676
CNZ57 ^d	<i>Cocos nucifera</i>	F:AGTGACAGCTCAAAGCAGTAT R:GTGGAGTACACAACCTATGGA	97-131	58	4	0.580	0.666	ns	-0.565
CNZ04 ^c	<i>Cocos nucifera</i>	F: CCTATTGCACCTAAGCAATTA R: TGATTTTCGAAGAGAGGTC	79-136	56	4	0.560	0.792	***	-0.392
CNZ50 ^d	<i>Cocos nucifera</i>	F: TCGACTAAGTGTGTCCATTC R: ATCCATCCAGGATCCCAATAT	93-100	60	3	0.542	0.703	*	-0.187
EE54 ^e	<i>Euterpe edulis</i>	F: CATGTATCTAAGGAACAAGG R: CTGTGCTCTCTCATTCTCA	128-137	58	3	0.638	0.523	ns	0.087
Mean					4.1	0.579	0.780		-0.420

Note: "F" and "R" refer to nucleotide sequences of forward and reverse SSR primers, respectively. Ta: optimal annealing temperature (°C) of each primer pair; Na: total number of alleles for each SSR locus; He: expected heterozygosity; Ho: observed heterozygosity; HWE = Hardy-Weinberg equilibrium, where ns refers to non-significant, whereas asterisks refer to the level of significance (*P < 0.05, **P < 0.01, ***P < 0.001) in HWE equilibrium based on Chi-square statistics; F_{IS}: inbreeding coefficient.

^aSSR primers developed by BILLOTTE et al. (2004).

^bSSR primers developed by RODRIGUES et al. (2004).

^cSSR primers developed by RIVERA et al. (1999).

^dSSR primers developed by PERERA et al. (1999).

^eSSR primers developed by GAIOTTO et al. (2001).

It is noteworthy to mention that *Syagrus harleyi* is endemic to a highly overlooked biome that has been facing accelerated processes of deforestation, landscape fragmentation, climatic changes, and recurrent fire episodes. Therefore, our results are promising for a better understanding of the population dynamics of this palm tree. For instance, by including other populations along the natural range of *S. harleyi* (using the same primer set), it will be possible to estimate levels of gene flow, population structure, and genetic differentiation, thereby providing essential information for the conservation and/or proper management of this palm tree from Diamantina Plateau in Brazil.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

Research data is available in the repository: <<https://docs.google.com/document/d/10sb1mJmOjzt-uIG9q3s-7wG76fLKB4wr/edit?usp=sharing&ouid=111170768834321990445&rtopof=true&sd=true>>.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

This study did not use artificial intelligence.

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