GMR

Transferability of microsatellite markers in S*yagrus coronata* (Mart.) Becc. (Arecaceae), an iconic palm tree from the Brazilian semiarid region

R.R. Simplicio[1], D.G. Pereira[2] and A.M. Waldschmidt[1]

[1]Departamento de Ciências Biológicas,
Universidade Estadual do Sudoeste da Bahia, Jequié, BA, Brasil
[2]Departamento de Química e Exatas,
Universidade Estadual do Sudoeste da Bahia, Jequié, BA, Brasil

Corresponding author: R.R. Simplicio
E-mail: renatasimpliciouesb@gmail.com

Genet. Mol. Res. 16 (2): gmr16029699
Received March 12, 2017
Accepted May 5, 2017
Published June 29, 2017
DOI http://dx.doi.org/10.4238/gmr16029699

ABSTRACT. The licuri palm *Syagrus coronata* plays a key role in the ecology and economy of Brazilian semiarid region. Nonetheless, genetic data about populations of this species are absent even though the intensive and uncontrolled exploitation since colonial periods has threatened the sustainability and viability of licuri populations. Therefore, we attempted to test the efficacy of transferability of microsatellite loci isolated from three palm tree species to *S. coronata* to analyze the population of this species throughout their range. A set of 19 heterologous microsatellite loci was tested in three native populations of *S. coronata* from the State of Bahia, northeastern Brazil, which amplified using distinct annealing temperatures (50°-60°C). Based on the 10 most polymorphic loci, the selected populations exhibited a mean number of alleles per locus of 9.8, and high genetic

diversity values since the expected heterozygosity ranged from 0.573 to 0.754, while the observed heterozygosity varied from 0.785 to 1.000. In conclusion, the tested loci are transferrable and highly efficient to population studies in *S. coronata*, thus minimizing the lack of species-specific loci to the genetic monitoring of licuri populations.

Key words: Arecaceae; Caatinga; Conservation genetics; SSR

INTRODUCTION

Several palm tree species have been widely exploited because of their useful resource to human communities; this is the case of the licuri palm *Syagrus coronata* (Mart.) Becc. (Arecacea), a species of biological and social-economic relevance in the semiarid region of Brazil, being widespread from the northern portion of the State of Minas Gerais, central and eastern parts of Bahia, Sergipe, Alagoas to southern Pernambuco (Drumond, 2007).

Because of their high energetic fruits and high resistance to drought periods, the licuri palm has been historically harvested as a food source. Therefore, the large-scale exploitation of this palm since colonial periods has reduced or extinguished populations along their original range. However, genetic studies in populations of the licuri palm are virtually unavailable, even though their spatial distribution in the semiarid landscape is well known and *S. coronata* has been a target of biofuel production (Ramalho, 2008).

In this sense, the simple sequence repeats (SSR) or microsatellites stand out as one of the most useful molecular markers to assess gene flow, genetic diversity, and population structure in vegetal species (Xiao et al., 2014). These codominant markers comprise highly polymorphic in tandem repeats (1 to 6 nucleotides) widespread throughout the genome of eukaryotes, particularly in non-coding regions. In the face of the fast-evolving environmental and economic crisis, efficient tools for the diagnosis of vulnerability status of species that play a key role in ecosystems, such as the licuri palm, should be developed. Since species-specific microsatellite markers in *S. coronata* are absent, the transferability of heterologous loci represents a viable alternative to infer gene flow among populations and the selection of priority areas for conserving genetic diversity and evolutionary potential of vulnerable species. Usually, the success of amplification using heterologous primers depends on previously reported data about related species (e.g., congeneric taxa) because of the occurrence of homologous microsatellite regions (Cota et al., 2012).

Therefore, the goal of the present study was to test the efficiency of transferability of SSR primers developed for three palm species - *Cocos nucifera* L. (Arecaceae), *Bactris gasipaes* Kunth (Arecaceae), and *Euterpe edulis* Mart. (Arecaceae) - for the amplification of microsatellite loci in *S. coronata* (Mart.) Becc. (Arecaceae). This result can be useful to further population genetic studies in the licuri palm, thus contributing to the genetic conservation and management of this species.

MATERIAL AND METHODS

Sampling

Samples of *S. coronata* were obtained from three municipalities in the State of Bahia,

northeastern Brazil: Serrinha, Cocos, and Jequié. Each population was composed of 10 specimens, totaling 30 samples. A minimum distance of 10 m among individuals was adopted to collect samples within each locality. The inflorescences were deposited in the Herbarium at Universidade Estadual do Sudoeste da Bahia (HUESB).

DNA extraction

About 100 g tissue from young leaves of *S. coronata* was used for the DNA extraction, following the protocol developed by Arruda (2014). The amount and integrity of extracted DNA were verified by electrophoresis on 0.8% agarose gel, stained with GelRed® and photodocumented under UV using the L-pix® system.

Amplification and analysis of SSR loci

A set of 19 microsatellite primers previously developed for *C. nucifera* (Perera et al., 1999; Rivera et al., 1999), *B. gasipaes* (Billotte et al., 2004; Rodrigues et al., 2004), and *E. edulis* (Gaiotto et al., 2001) was tested in DNA samples of *S. coronata*. The amplification of SSR loci via PCR (polymerase chain reaction) was carried out according to Peters et al. (1998).

Each reaction comprised 1 µL 10X buffer (BioTools), 0.2 µL of each primer at 20 µM, 0.3 µL $MgCl_2$ at 50 mM, 0.4 µL dNTP at 2.5 mM, 5 U 0.1 µL Taq polymerase, 10 ng template DNA, and ultrapure water to a final volume of 10 µL. The PCR was performed using a thermocycler (Applied Biosystems) with the following program: an initial denaturation step at 94°C for 3 min, followed by 40 cycles of denaturation at 92°C for 30 s, annealing according to the tested temperature gradient (50°, 52°, 54°, 56°, 58°, and 60°C) for 1 min and extension at 72°C for 30 s, plus a final extension step at 72°C for 5 min.

The amplification products were genotyped using a MegaBace-1000 (GE Healthcare, Buckinghamshire) automated sequencer. The size of fragments was established based on genotyping peaks after comparison with the ET550R size marker in the MegaBace Fragment Profiler v. 2.2 software (GE Healthcare). A preliminary analysis was carried out to identify and correct putative genotype errors using Micro-Checker v. 2.2.3 (Peakall and Smouse, 2012).

The 10 most polymorphic SSR loci amplified from the licuri palm were evaluated using the GenAlEx software (Peakall and Smouse, 2012) by estimating the expected (H_E) and observed (H_O) heterozygosity values, the number of alleles per locus (N_A), and deviations from the Hardy-Weinberg equilibrium (HWE) for each primer per sampled population.

RESULTS

The 19 tested loci were free of null alleles and properly amplified at distinct annealing temperatures, ranging from 50° to 60°C (Table 1). Among them, 10 markers were the most polymorphic ones and isolated from *C. nucifera* and *B. gasipaes*.

The genetic analysis of these 10 polymorphic loci in *S. coronata* revealed 5 to 12 N_A and high levels of genetic diversity (H_E = 0.573-0.754). Similarly, the H_O values varied from 0.785 to 1.000, being higher than the H_E (Table 2). Significant HWE deviation was observed for the loci CNZ50 and CNZ23 in the population from Cocos municipality ($P < 0.001$), as well as for CNZ23, Bg02-09, and mBgCIR066 in samples from Jequié and CNZ50 in the population from Serrinha ($P < 0.005$).

Table 1. Sequences of tested heterologous loci in *Syagrus coronata*, with their respective fragment size in bp and best annealing temperature (Ta).

Primers	Sequence (5'-3')	Fragment size	Ta (°C)
Bg02-09	F: CGCAGCAGCAGCAATAAATA	170-174	60
	R: TCCAGCAACTTTCAGTCGAG		
Bg02-10	F: ATTGGGTCCAGATCCTCTTT	150-176	52
	R: GTGGCACACATGGGGTTC		
CNZ03	F: CATCTTTCATCATTTAGCTCT	132-140	60
	R: AAACCAAAAGCAAGGAGAAGT		
CNZ04	F: CCTATTGCACCTAAGCAATTA	128-158	56
	R: TGATTTTCGAAGAGAGGTC		
CNZ10	F: CCTATTGCACCTAAGCAATTA	129-169	50
	R: ATGATTTTCGAAGAGAGGTC		
CNZ13	F: TATGCTATTCACTCATTTTTCG	138-178	60
	R: ACTCTGTTTCACGATCAAAAA		
CNZ23	F: ATCAAAACATGACACCGTAAC	133-163	58
	R: CTGATAGATGACAAGGTGTGG		
CNZ26	F: CTAGGCTCCCCATGTGTTTTT	216-240	58
	R: CACTGCTGTTGTACACCTCCA		
CNZ44	F: CATCAGTTCCACTCTCATTTC	139-151	60
	R: CAACAAAAGACATAGGTGGTC		
CNZ50	F: TCGACTAAGTGTTGTCCATTC	114-120	54
	R: ATCCATCCAGGATCCCAATAT		
CNZ51	F: CTTTAGGGAAAAAGGACTGAG	138-150	60
	R: ATCCATGAGCTGAGCTTGAAC		
CNZ57	F: AGTGACAGCTCAAAGCAGTAT	99-105	50
	R: GTGGAGTACACAACCTATGGA		
CAC2	F: AGCTTTTTCATTGCTGGAAT	229-243	50
	R: CCCCTCCAATACATTTTTCC		
mBgCIR053	F: TTCAGTTAAGACCACCTATCA	148-176	56
	R: ACGAAGAAATCGAACCATAC		
mBgCIR058	F: TTTGATACCCCAGAGAGA	243-289	60
	R: AGCGAGAAACACGAATAC		
mBgCIR066	F: GCATGTTGCATTGACTA	253-257	54
	R: GAATCCTGGTTCAGATACT		
mBgCIR091	F: CAAGAACAGGCTCAGTCTA	200-212	60
	R: TGCAATCAACCCAAGAT		
EE41	F: CCTTGCAGTTTATGGCTACG	114-122	52
	R: CCATTGAGAGGGAATGAGGT		
EE54	F: CATGTATCTAAGGAACAAGG	113-115	60
	R: CTGTGCTCTCTCATTCTCA		

Table 2. Genetic diversity parameters (number of alleles per loci - N_A; expected heterozygosity - H_E; and observed heterozygosity - H_O) in *Syagrus coronata* based on 10 transferrable heterologous loci.

Locus	N_A	H_E	H_O
mBgCI053	12	0.741	0.926
mBgCI066	10	0.697	0.911
Bg02-10	11	0.754	0.932
Bg02-09	12	0.752	0.833
CNZ03	9	0.748	0.785
CNZ23	12	0.753	0.969
CNZ44	8	0.704	1.000
CNZ50	10	0.721	0.923
CNZ51	5	0.573	0.836
CNZ57	9	0.722	0.989
Mean	9.8	0.716	0.910

DISCUSSION

Because of the high species diversity and the costs of developing specific microsatellite markers, the transferability of heterologous primers is a suitable alternative to studies of population genetics in biodiversity hotspots (Barbará et al., 2007). This approach has been

successfully used in both vegetal and animal groups, such as pollinators (Viana et al., 2011) and dispersers.

Indeed, the efficient transferability of the 19 tested SSR primers to amplify microsatellite loci in *S. coronata* corroborates the applicability of heterologous primers to genetic studies in palm trees, thus allowing refined population analysis in licuri palms. Among the heterologous primers amplified in *S. coronata*, 11 were originally developed for *C. nucifera* (Perera et al., 1999; Rivera et al., 1999). This result could be related to the close phylogenetic relationship between the genera *Syagrus* and *Cocos* within the subtribe Attaleinae, as revealed by the analysis of seven genes encoding specific transcription factors in plants (Meerow et al., 2009). Closely related species and transferability of SSR loci can be higher than 60% (Barbará et al., 2007).

Moreover, the 10 selected microsatellite loci showed high values of H_E (Table 2), indicating high genetic diversity that should be important to the maintenance and adaptability of natural populations. Nonetheless, some loci exhibited increased H_O values (near or equal to 1.0), such as CNZ23 ($H_O = 0.969$) with significant deviation from HWE ($P < 0.005$). Thus, in spite of the high values of genetic diversity, the deviation from HWE indicates the influence of distinct evolutionary forces, including the putative role of genetic drift by population fragmentation (Frakham et al., 2008), what should be further analyzed.

Similarly, the transferability of 31 SSR markers described for *Passiflora edulis* Sims and *Passiflora alata* Dryander (Passifloraceae) allowed evaluating the genetic diversity in *Passiflora setacea* D.C. with views to conservation and genetic improvement (Pereira et al., 2015). These authors also reported high values of H_O with the significant deviation ($P < 0.01$) from HWE for all loci. Within *Citrus*, Cristofani-Yaly et al. (2011) obtained 100% efficiency in cross-species amplification using 24 SSR loci. These reports reinforce that genetic studies in species of great economic and ecological importance can benefit from the application of heterologous microsatellite primers.

Even though *S. coronata* is a key element to the biodiversity of caatinga, a threatened biome typical of the Brazilian semiarid region, little is known about its population structure. In fact, most genetic studies are focused on other palm species such as *Elaeis guineenses* Jacq. (Arecaceae) (Austin et al., 2015) and *Syagrus romanzoffiana* (Cham.) Glassman (Arecaceae) (Giombini et al., 2013). Therefore, the results from the present study provided a large number of microsatellite loci suitable for population studies in *S. coronata*. Based on the transferability of heterologous SSR primers, the levels of gene flow and genetic structure can be established in licuri palm populations, which are useful to the conservation and *in situ* or *ex situ* management of this iconic species from caatinga.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to “Programa de Formação de Recursos Humanos - Petrobras” (#PRH-PB 211) and “Fundação de Amparo à Pesquisa da Bahia” (FAPESB) for the financial support and the scholarship on behalf of R.R. Simplicio, and to the Herbarium at Universidade Estadual do Sudoeste da Bahia (HUESB) for the assistance in species identification.

REFERENCES

Arruda SR (2014). Diversidade e estrutura genética de *Mimosa tenuiflora* (Wild.) Poir.: Importante recurso florestal do semiárido brasileiro. Master's thesis, UESB, Jequié.

Austin KG, Kasibhatla PS, Urban DL, Stolle F, et al. (2015). Reconciling oil palm expansion and climate change mitigation in Kalimantan, Indonesia. *PLoS One* 10: e0127963. https://doi.org/10.1371/journal.pone.0127963

Barbará T, Palma-Silva C, Paggi GM, Bered F, et al. (2007). Cross-species transfer of nuclear microsatellite markers: potential and limitations. *Mol. Ecol.* 16: 3759-3767. https://doi.org/10.1111/j.1365-294X.2007.03439.x

Billotte N, Couvreur T, Marseillac N, Brottier P, et al. (2004). A new set of microsatellite markers for the peach palm (*Bactris gasipaes* Kunth); characterization and across-taxa utility within the tribe Cocoeae. *Mol. Ecol. Notes* 4: 580-582. https://doi.org/10.1111/j.1471-8286.2004.00741.x

Cota LG, Moreira PA, Menezes EV, Gomes AS, et al. (2012). Transferability and characterization of simple sequence repeat markers from *Anacardium occidentale* to *A. humile* (Anacardiaceae). *Genet. Mol. Res.* 11: 4609-4616. https://doi.org/10.4238/2012.October.17.7

Cristofani-Yaly M, Novelli VM, Bastianel M and Machado MA (2011). Transferability and Level of Heterozygosity of microsatellite Markers in *Citrus* Species. *Plant Mol. Biol. Report.* 29: 418-423. https://doi.org/10.1007/s11105-010-0241-x

Drumond MA (2007). Licuri *Syagrus coronata* (Mart.) Becc. Petrolina. Embrapa Sémi-Árido, Pernambuco.

Frakham R, Ballou JD and Briscoe DA (2008). Fundamentos da Genética da Conservação. Sociedade Brasileira de Genética, São Paulo.

Gaiotto FA, Brondani RPV and Grattapaglia D (2001). Microsatellite markers for Heart of Palm - *Euterpe edulis* and *E. oleracea* Mart (Arecaceae). *Mol. Ecol. Notes* 1: 86-88. https://doi.org/10.1046/j.1471-8278.2001.00036.x

Giombini MI, Tosto DS and Bravo SP (2013). Characterization of 20 microsatellites in the Neotropical palm *Syagrus romanzoffiana* (Arecaceae) identified by cross-amplification from across genera. *Mol. Ecol. Notes* 12: 1196-1197.

Meerow AW, Noblick L, Borrone JW, Couvreur TLP, et al. (2009). Phylogenetic analysis of seven WRKY genes across the palm subtribe Attaleinae (Arecaceae) [corrected] identifies Syagrus as sister group of the coconut. *PLoS One* 4: e7353. https://doi.org/10.1371/journal.pone.0007353

Peakall R and Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* 28: 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Pereira DA, Gaiotto FA, Corrêa RX and Oliveira AC (2015). Heterologous primer transferability and access to microsatellite loci polymorphism in "somnus" passion fruit tree (*Passiflora setacea* DC). *Biotemas* 3: 51-56.

Perera L, Russell JR, Provan J and Powell W (1999). Identification and characterization of microsatellite loci in coconut (Cocos nucifera L.) and the analysis of coconut populations in Sri Lanka. *Mol. Ecol.* 8: 344-346.

Peters JM, Queller DC, Fonseca VL and Strassmann JE (1998). Microsatellite loci for stringless bees. *Mol. Ecol.* 7: 784-787.

Ramalho CI (2008). Estrutura da vegetação e distribuição espacial do licuri *Syagrus coronata* (Mart) Becc. em dois municípios do Centro Norte da Bahia, Brasil. Universidade Federal de Pernambuco, Areia.

Rivera R, Edwards KJ, Barker JHA, Arnold GM, et al. (1999). Isolation and characterization of polymorphic microsatellites in *Cocos nucifera* L. *Genome* 42: 668-675. https://doi.org/10.1139/g98-170

Rodrigues DP, Vinson C, Ciampi AY, Farias IP, et al. (2004). Novel microsatellite markers for *Bactris gasipaes* (Palmae). *Mol. Ecol. Notes* 4: 575-576. https://doi.org/10.1111/j.1471-8286.2004.00739.x

Viana MVC, Miranda EA, de Francisco AK, Carvalho CAL, et al. (2011). Transferability of microsatellite primers developed for stingless bees to four other species of the genus *Melipona. Genet. Mol. Res.* 10: 3942-3947. https://doi.org/10.4238/2011.November.22.11

Xiao Y, Zhou L, Xia W, Mason AS, et al. (2014). Exploiting transcriptome data for the development and characterization of gene-based SSR markers related to cold tolerance in oil palm (*Elaeis guineensis*). *BMC Plant Biol.* 14: 384-396. https://doi.org/10.1186/s12870-014-0384-2