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Transfer of microsatellite markers from other Arecaceae species to *Syagrus romanzoffiana* (Arecaceae)

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Genet. Mol. Res. 18 (3): gmr18183
Received October 19, 2018
Accepted April 05, 2019
Published July 18, 2019
DOI http://dx.doi.org/10.4238/gmr18183

ABSTRACT. The species *Syagrus romanzoffiana*, a native palm tree of South America, is widely distributed and well adapted to southern Brazil. It is an important economic, cultural, and ecological resource, being the preferred food of diverse animal species, involving complex ecological relationships. However, despite numerous molecular studies on native plants, specific molecular markers, such as expressed sequence tag-simple sequence repeats (EST-SSRs), are lacking, and there are few SSR markers for this species. Molecular data about individuals and their populations offers new perspectives for management, conservation, and economic exploitation. Here we tested 21 microsatellite loci known from three species of the family *Arecaceae* in three native populations of *S. romanzoffiana* in the state of Rio Grande do Sul, in southern Brazil. Among the 21 primer pairs tested, 12 produced an amplification product. Five loci were chosen and subjected to diversity tests. The populations presented between three and nine alleles per locus, and acceptable genetic diversity values; the expected heterozygosity ranged from 0.460 to 0.864, while the observed heterozygosity ranged from 0.100 to 1.000. We conclude that various microsatellite markers developed for other species of the family *Arecaceae* are suitable for molecular analysis of *S. romanzoffiana* populations.

Key words: Jerivá; Molecular marker; SSR

INTRODUCTION

The species *Syagrus romanzoffiana*, belonging to the *Arecaceae* family and locally known as Coqueiro Jerivá, is an important example of Brazilian flora (Lorenzi et al., 2010; Noblick, 2017). Exclusively found in South America, this plant grows throughout the country, from the south of Bahia to the extreme south of Brazil (Reitz et al., 1974; Noblick and Lorenzi, 2010). *Syagrus romanzoffiana* is a palm tree that has probably been used since the early human occupation of the continent, as a source of food and fermented beverages; in the construction of houses, handicrafts, and pigments; as a fuel; and in medicinal uses in traditional culture, as deduced from archaeological records (Raupp et al., 2007; Zambrana et al., 2007; Bonomo and Capeletti, 2014). It is a wild species indigenous to South America, but owing to the ease with which it can be cultivated, it is one of the most widely used ornamental plants in urban settings in southern and southeastern Brazil (Reitz et al., 1974; Lorenzi et al., 2010).

Syagrus romanzoffiana has pinnate leaves that range from 2 to 3 m in length, with leaflets that can reach up to 25 cm in length. Pinnate leaves are characterized by the irregular arrangement of leaflets in the raque, which is responsible for the voluminous fan-like appearance of these leaves. This palm can reach up to 25 m in height and has a single, cylindrical, grayish-colored stripe. As a monóica plant, characterized by panicle-like inflorescence spikes, the flowers of *S. romanzoffiana* are dioecious and are light-yellow in color. The fruits of this tree are globose-oval drupe with fibrous pulp, yellow-orange in color, with a sweet taste, with up to 800 units per panicle. Besides Brazil, *S. romanzoffiana* is also indigenous to Paraguay, Uruguay, and Argentina. This tree has a variety of popular names; among the most interesting in Brazil include: Baba-de-boi, Coqueiro, Coqueiro-jerivá, Coquinho-de-cachorro, Jeriba, Coco-juvena, Coco-de-catarro, Brejaúba, Gerivá, Jiruvá, Palmito-amargoso, and Imbury-de-cachorro. Argentina, Uruguay, and Paraguay also have unique names for this tree, including Pindó, Palma-del-monte, and Pindó-das-missiones. In the United States, *S. romanzoffiana* is popularly known as the Queen Palm (Reitz et al., 1973; Lorenzi et al., 2010; Noblick, 2017) (Figure 1).

Syagrus romanzoffiana has a fundamental ecological role, being the preferred food of numerous species of mammals, birds, and insects, mainly because of its nutritional characteristics and wide spectrum of fruiting. Thus, it constitutes an important component of natural ecosystems, being integrated through interactions established with its dispersers and pollinators (Terborgh, 1986; Siqueira, 1989; Zona and Henderson 1989; Souza et al., 1994; Carvalho, 2006; Galetti et al., 2013).

Considering the destruction of the natural habitat and important ecological role played by the Jerivá palm tree, it is necessary to develop conservation strategies for this species. This is especially important since this species plays a key role in maintaining a healthy ecosystem (Roesch et al., 2009; Laindorf et al., 2018).

Molecular studies are required to provide data on diversity, conservation status, gene flow, and the molecular ecology of the species (Carvalho, 2006; Lorenzi et al., 2010; Bonomo and Capeletti, 2014). Obtaining microsatellite markers, also known as simple sequence repeats (SSRs), is vital for genetic diversity studies (Sebbenn et al., 2011). In addition, markers of gene regions can be useful for genetic improvement, as they are associated with the expression of phenotypes, which may be of economic interest (Gupta et al. 2003). Microsatellites are very short DNA sequences of 1–6 base pairs, repeated in

tandem, which are detected by polymerase chain reaction (PCR) using specific primers (Litt and Luty, 1989; Weber and May 1989; Zucchi et al., 2003). These markers allow the generation of a large amount of information about the genetic identity, diversity, gene frequency, and phylogenetic relationships of the genetic resources of a certain germplasm, including the genetic conditions of a natural population. This information is useful for conservation strategies of genetic resources, as well as aiding in the development of conservation strategies more in keeping with the real needs of the species (Powell et al., 1996; Bittencourt and Sebbenn, 2009; Victoria et al., 2011).

Figure 1: Habit of the palm tree Jerivá. In detail the fruit features of this palm species.

Microsatellite molecular markers generally constitute information necessary to estimate the various genetic parameters of interest (Ferreira and Grattapaglia, 1998). They are more polymorphic in comparison with other markers and have been considered as most informative in estimates of population genetic parameters (Kalia et. al, 2011)

To overcome the lack of expressed sequence tag-SSRs (EST-SSRs) and the scarcity of SSR molecular markers specific to the species of interest, there is the possibility of transfer of primers developed in related species, a technique that has obtained significant success within the family Arecaceae (Kuleung et al., 2004; Manju et al., 2017). Alternatively, these markers can be found in online databases, where the sequences are deposited and are available.

The objective of this work was, therefore, to investigate the transfer of SSR and EST-SSR microsatellite markers, specifically for the study of *S. romanzoffiana.* A member of the Arecaceae family, thereby allowing molecular studies of this species.

MATERIAL AND METHODS

Sampling

Leaf samples of 30 individual *S. romanzoffiana* trees were obtained from three different regions in the state of Rio Grande do Sul, southern Brazil. Each region sampled corresponds to 10 individuals. Collection expeditions began in 2016 and only adult palm trees identified by the presence of reproductive structures or scars were selected. Individual samples were identified in the field by the authors, using the dichotomous key of Noblick (2017). The individual trees sampled belong to natural populations, located in rural areas and private properties. The collection is stored in an Ultrafreezer at -80°C, deposited under number 008, at the Herbarium Germplasm Bank of Edgar Bruno Irgang (HBEI) at the Federal University of the Pampa.

DNA extraction

Total DNA was isolated from the young leaves of the sampled individuals using a Qiagen Plant MiniKit® kit (Hilden, Germany), following the manufacturer's instructions. The amount and quality of the DNA were evaluated using a NanoVue™ Plus Spectrophotometer.

Selecting SSR and EST-SSR Markers

For the transfer study, we chose 21 pairs of SSR marker primers (Table 1. List of primers). These markers comprised 10 genomic SSRs originally developed for *Livistona chinensis* and *Cocos nucifera*, with transfer results to *Butia odorata*, reported by Mistura et al. (2012). The other 11 primers were for EST-SSRs identified from sequences available from the GenBank database (NCBI) for the Arecaceae family. Through a BLAST search, it was possible to identify the sequences corresponding to the species *Elaeis guineensis* and *Phoenix dactylifera*.

Prospecting EST-SSR markers

The total EST data for the Arecaceae family, available in the GenBank database, were downloaded in FASTA format. The resulting sequence was submitted to the DNA sequence assembly program (Huang et al., 1999) and considered in the SSR locator software (Da Maia et al., 2008) with minimum parameters of six replicates of the motif. The most frequent data, types, and reasons were recorded. From these data, 11 markers were selected to be synthesized. To confirm which species belonged to the selected markers, a Blast search was performed. It was not possible to identify the species for two primers.

PCR survey of SSR loci

The PCR reactions were adapted from Geethanjali et al. (2017), using 2 µL of *S. romanzoffiana* DNA, 1 µL of forward and reverse primers; 1.5 µL of buffer (10×); 1 µL $MgCl_2$ (5U / µL); dNTP 1 µL (100 mM); 0.2 µL Taq Polymerase buffer (5U / µL); and 7.3

µL of ultra pure water (Milli-Q), to make a total reaction of 20 µL. Amplifications were performed on a BioRad C1000 Touch™ thermocycler, programmed for an initial stage of DNA denaturation at 94°C for five min; followed by 35 cycles of 94°C for 30 s for denaturation, 1 min at the annealing temperature (Ta) step, in which the gradient used varied between 50 and 60°C, and 1 min for extension at 72°C; with a final extension at 72°C for 10 min, before being held at 4°C.

Table 1. Characteristics of 21 microsatellite loci transposed to *Syagrus romanzoffiana*.

Code	Locus	Sequence	T/NT	RT	PSA/PSS	TA	OS
SSR							
MSr01	LCS0012 AB435527	F:AACTGCAGGAACAAAGACGATT R:TCACGTCCACGTATTCGTACTC	T	(TC)18	246–254 / 260	45 - 50	*Livistona chinensis*
MSr02	LCS0015 AB435528	F:CATGGAATTGTAATCCCCACTT R:TATCCACTTGTCGGAGTTTTCC	T	(CT)19	157–198 /200	45 - 50	*Livistona chinensis*
MSr03	LCS0037 AB435530	F:CATAGGCAGTCACAGATGGTTT R:CCAGCTAATTTCCCCTTCTTTT	T	(GA)14	269–291 / 290	45 - 50	*Livistona chinensis*
MSr04	LCS0135 AB435533	F:CTCTAGCTGGGCTCTTCCTATG R:GGTGTGAATGTGCAGGATAGAA	T	(CT)19	89–105 / 100	45 - 50	*Livistona chinensis*
MSr05	LCS0187 AB435534	F:ATGTTTTCTTGGTGGGTTTTTG R:TCCCTTTCTCTTGCTTTCAATC	T	(CT)15	228–252 / 280	45 - 50	*Livistona chinensis*
MSr06	LCS0006 AB435526	F:TGCAATAGCTTCGGATATGAAA R:ACCATGCGAAACTGTCTAAGGT	T	(CT)24	240–266 / 140	45 - 50	*Livistona chinensis*
MSr07	LCS0240 AB435535	F: CTTACCTTGACGAGTCGCCTAC R:CGGCGAAATGTCTTCTAACAAT	NT	(TC)17	195-213	45 - 50	*Livistona chinensis*
MSr08	CNZ01	F:ATGATGATCTCTGGTTAGGCT R:AAATGAGGGTTTGGAAGGATT	NT	NP	122	55	*Cocos nucifera*
MSr09	CNZ04	F:TATATGGGATGCTTTAGTGGA R:CAAATCGACAGACATCCTAAA	NT	NP	162	53	*Cocos nucifera*
MSr10	CNZ05	F:CTTATCCAAATCGTCACAGAG R:AGGAGAAGCCAGGAAAGATTT	NT	NP	163	53	*Cocos nucifera*
EST-SSR							
MSr26		F:TTTACAAAAAGGAACATCAT R:TAAATCTGATGGAAAGTCAT	T	(AG)16	156 / 180	50 - 55	*Phoenix dactylifera* *Elaeis guineensis*
MSr27		F:AGCAAGGTCAGCGAA R:ACGAGAAGGGAACAAAC	T	(TC)17	100 / 290	50 - 55	*Phoenix dactylifera* *Elaeis guineensis*
MSr28		F:TGCTTCTTATTGAGAGGTAA R:CAGAAGTGAGTAGGAGACAC	T	(AG)17	110 / 105	50 - 55	*Phoenix dactylifera* *Elaeis guineensis*
MSr29		F:ATTCATTATTCAACACCAAC R:GGTCTCTCTCTCTCTCTCTC	T	(GT)19	260 / 200-300	50 - 55	*Phoenix dactylifera* *Elaeis guineensis*
MSr30		F: CAATATTTCTGTGATGATGA R: AGAACAGAAACAATGACAAG	NT	(GA)54	257	50	*Phoenix dactylifera*, *Elaeis guineensis*
MSr31		F: CTCTCCCTTCTTCTACTCTC R: GCATCTAAAAACAATAGGAA	NT	(AG)18	212	50	*Phoenix dactylifera* *Elaeis guineensis*
MSr32		F: TTATGGAATGTGGTAGTAGG R: GACTGAATAGGGAATTGAA	NT	(TC)14	198	50	*Phoenix dactylifera* *Elaeis guineensis*
MSr33		F: CTACCATAGATCACCAACC R: TAGATCCATTTATTCCGATA	NT	(CT)14	270	50	*Elaeis guineensis*
Msr34		F:ATTATACTGAGCTTGTGGAA R:TTTTAAAGACTATTTGGGTG	T	(CT)14	260 / 200	50 - 52	GenBank - *Arecaceae*
MSr35		F: TATCCTTAAGCGTAAAAGAA R: CGGATTATCTCTAGATCTGT	NT	(GA)16	275	50	GenBank - *Arecaceae*
MSr36		F:ATAAGCTTCTCTAATCCTCC R: CGACACGTACTTGTAAGC	T	(AG)50	241 /200	50 - 55	*Phoenix dactylifera*

*Transferability or no Transferability (T/NT), *Repeat type (RT) *Product size in Arecaceae species / product size in S. romanzoffiana (PSA/PSS) *Annealing temperature (TA) *Not Published (NP) * Original species (OS).

The annealing temperatures varied between 50 and 55ºC, with 51ºC being chosen as the optimum temperature. The PCR products were separated on 3% Agarose gel, diluted in 1×Tris-borate-EDTA (TBE) buffer, with loading of 80 Volts, and visualized using the dye Gel Red (Invitrogen) in comparison with 100 bp ladder markers (Norgen). Matrices were generated using the program LPix EX-2.6-PGR (Locus Biotecnologia, Cotia, Brazil), which were evaluated for the size of the fragments and the pattern of bands observed in the gel images.

Data analysis

The five loci that were amplified most successfully were evaluated for the genetic diversity values of the 30 individuals of *S. romanzoffiana*, using GeneAlex (Genetic Analysis in Excel) software (Peakall and Smouse, 2012). Expected heterozygosity (*He*), observed heterozygosity (*Ho*), the number of alleles per locus (*A*), the effective number of alleles (A_E), and the inbreeding coefficient (*F*) were estimated.

RESULTS

The 1,563 pairs of EST-SSR primers were obtained through the SSRLocator software and the database for Arecaceae in GenBank. The analyzed database initially contained 55,403 contigs, with a GC content of 43.1% and a total of 13,056,326 bp; after the elimination of redundancy, we obtained 7,650 contigs, with a GC content of 42.7% and 6,196 bp. The database and the SSRlocator also allowed for the detection of 1935 motifs, of which 429 were dinucleotide, 975 were trinucleotide, 346 were tetramers, 82 were pentanucleotide, and 103 were hexanucleotide. The most abundant repetitive motifs found were GA/TC and AAG/GAG for the dinucleotide and trinucleotide motifs, respectively.

Among the 21 primers pairs tested, 12 successfully amplified a product from *S. romanzoffiana*. Of the 12 primer pairs that were successful, six were genomic SSRs and the other six were EST-SSRs. The amplification patterns that were generated can be considered valid because of the few nonspecific products amplified by primers MSr02, MSr03, MSr04, MSr06, MSr29, MSr34, and MSr36; and the absence of nonspecific bands in amplified by primers MSr01, MSr05, MSr26, MSr27 and MSr28.

The amplicons for the transferred markers showed different sizes compared with those reported in the original species. In addition, the annealing temperature was different, being efficient at 51°C for all markers (Table 1). The five markers with sufficient amplification (MSr01, MSr02, MSr03, MSr27, and MSr28) were chosen for diversity testing.

Genetic analyzes of the five loci were informative, revealing that the number of alleles per locus (A) varied between one and nine, and the effective number of alleles (*Ae*) varied between 1.852 and 8.000. The observed heterozygosity (*Ho*) was low, presenting values between 0.100 and 0.500, and the expected heterozygosity (*He*) ranged from 0.460 to 0.875. The fixation Index in Guaíba population was 0.862, Coastal population 0.784 and Uruguay population 0.653 (Table 2).

Table 2. Genetic diversity parameters for tree palm *Syagrus romanzoffiana* based on five transferrable loci: Percentage of polymorphism per population (*% polym.*), number of alleles per locus (*A*), effective number of alleles (*Ae*), Observed Heterozygosity (H_O), Heterozygosity Expected (H_E), and Fixation Index (*F*).

Population	% polym.	Locus	A	Ae	H_O	H_E	F
Guaíba	100%	MSr01	5.00	3.84	0.100	0.740	1.000
		MSr02	6.00	5.158	0.143	0.806	0.823
		MSr03	9.00	7.364	0.444	0.864	0.486
		MSr27	5.00	4.167	0.100	0.760	1.000
		MSr28	6.00	5.556	0.100	0.820	1.000
		Multiloci	###	5.218	0.117	0.798	0.862
Litoral	80%	MSr01	5.00	3.846	0.100	0.740	1.000
		MSr02*	1.00	1.000	0.000	0.000	-
		MSr03	6.00	3.273	0.500	0.694	0.280
		MSr27	6.00	3.175	0.100	0.685	0.854
		MSr28	3.00	1.852	0.100	0.460	1.000
		Multiloci	###	2.629	0.120	0.516	0.784
Uruguai	100%	MSr01	8.00	7.143	0.100	0.86	1.000
		MSr02	8.00	8.000	0.204	0,78	0.643
		MSr03	5.00	2.723	0.38	0.633	0.407
		MSr27	4.00	4.000	0.1	0.750	1.000
		MSr28	3.00	1.976	0.1	0.494	1.000
		Multiloci	###	4.768	0.275	0.722	0.810

* Absence of polymorphism. Significance: ($P < 0.001$)

DISCUSSION

Palliyarakkal et al. (2011), looking for SSR sequences retrieved from public domains, reported a large quantity of microsatellite markers available for the Arecaceae family (38,086) when compared to our study (1563). Furthermore, the most frequent motifs were dinucleotide. This does not agree with our data, which show that trinucleotide motifs are the most abundant. This discrepancy can be attributed to the type of data filtering used in the analyses. That is, in the search of primers of this study we used a more selective filter, which resulted in a decrease in the total number of primers. Along with the increase in the quantity of available data in recent years, the pattern of the sequences may have changed, thus changing their frequency.

The marker transfer efficiency of 12/21 in our study shows a satisfactory transferability, considering that the species under test are not in the same genus. Saha et al. (2004) reported a transfer rate for EST-SSR markers of 66% in a study on grasses, which was considered high for close taxonomic relatives that share the same genus.

The efficiency of transfer in the our study was supported by the work of Mistura et al. (2012), who obtained an effective transferability of 40% for markers developed for *Cocos nucifera* transferred to *Butia odorata*. Fortes et al. (2016) recorded a high rate of 75% for transfer from the palms *Bactris gasipaes* and *Astrocaryum aculeatum* to *Astrocaryum vulgare*, which was expected in the second case because they share the same genus. Aberlenc-Bertossi et al. (2014) reported a success rate of 45% for the transfer of markers from *Cocos nucifera* to other species in the same family. Previously reported marker transfer records within the *Arecaceae* family support the efficiency of the transferability to *S. romanzoffiana* of the markers tested in the present study, especially since the species used are considered taxonomically distant (Mengistu et al., 2015)

The EST-SSR markers are not yet cited in the literature for the species. According to Castillo et al. (2008), these are very useful because they have a higher transfer rate compared with genomic SSRs, as well as being a better option for phylogenetic studies, EST-SSRs also serve as tools for studies of genetic diversity.

The size of the amplified products in the *S. romanzoffiana* species were different from those amplified in the original organisms, a phenomenon that has been noted previously (Castillo et al., 2008). Likewise, the broad amplification spectrum verified for the primers in the present study is reported by Ohtani and Yoshimaru (2014). In addition, the annealing temperatures (TA) need to be adjusted for six markers, which has been observed previously for the transfer of primers within the family Arecaceae (Fortes et al., 2016).

The results demonstrated the efficiency of the technique, opening up the possibility of using these SSRs and EST-SSR markers, in addition to those already existing for the species, to increase the coverage of the genome and the number of alleles to be explored, thus enriching the data for more accurate molecular analyses. These transferred markers will also increasing the efficiency of molecular analyses in this species, allowing faster molecular characterization at reduced cost.

Recently, Simplicio et al. (2017) reported that 19 SSR markers found in the Licuri palm were transferred from *Cocos nucifera*, *Bactris gasipaes*, and *Euterpe edullis*. As the Licuri palm (*Syagrus coronata*) shares the same genus as the Jerivá palm (*S. romanzoffiana*), the approach we have used in this study can also be applied to determine whether or not the same 19 SSR markers were also transferred to *S. romanzoffiana*.

ACKNOWLEDGMENTS

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Finance Code 001, the Conselho Nacional de Desenvolvimento Científico – CNPq (grant number 446234/2015-0), and from the Universidade Federal do Pampa – UNIPAMPA.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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