

Transferability and Characterization of Microsatellite Markers from *Byrsonima Cydoniifolia* A. Juss. (MALPIGHIACEAE) in Seven Related Taxa from Cerrado Biome Reveal Genetic Relationships

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Abstract

Byrsonima Rich. is one of the largest genera of the Malpighiaceae family, with 97 species occurrence in Brazil. In this study, 17 microsatellite markers previously developed and characterized in *Byrsonima cydoniifolia* A. Juss. were tested for seven related taxa. All species tested here are native to Brazil, and of these species four are endemic. Cross-amplification was successfully optimized with high efficiency for all species. Microsatellite markers panels ranged from 11 (64,8%) transferred markers in *B. viminifolia* to 6 (35.2%) in *B. umbellata*. All loci were genotyped for 16 individuals of each species, except for *B. viminifolia* (14 individuals), then the polymorphic loci were characterized. The total number of alleles per locus across tested species ranged from 5 (*B. linearifolia*) to 8 (*B. subterranea*) alleles. *B. umbellata* showed lower values of observed and expected heterozygosity ($H_o = 0.312$; $H_e = 0.436$) and *B. subterranea* presented the highest values ($H_o = 0.687$; $H_e = 0.778$). A greater number of microsatellite markers should be developed for *B. umbellata*, once that the markers set transferred is reasonably informative, due to the greatest genetic divergence between species. The microsatellite marker panels transferred to the species *B. intermedia*, *B. verbascifolia*, *B. laxiflora*, *B. subterranea*, *B. viminifolia* and *B. linearifolia* are very informative, with a high combined probability of exclusion of paternity ($Q \geq 0.976$) and the low combined probability of identity ($I \leq 9.91 \times 10^{-6}$), potentially suitable for future genetic-population studies, supporting strategies for maintaining the genetic diversity and for exploration of *Byrsonima* species as genetic resources.

Introduction

Byrsonima Rich., one of the largest genus belonging to the Malpighiaceae family, contains approximately 150 species, distributed from Mexico to South America. From these about 97 species occurs in Brazil, with a wide distribution in Cerrado Biome, popularly known as muricizeiro or murici [1] (Mamede and Francener, 2015). Rarely cultivated, *Byrsonima* species have an enormous agricultural economic potential, can be used in ornamentation, reforestation of degraded areas, and forage in times of lack of pasture [2] (Vieira et al., 2010). Leaves are used in folk medicine and in the tannery industry. Fruits and the oil extracted from the seeds are used in the food and pharmaceutical industry [3] (Almeida et al., 2011). Fruit extracts present a potent anti-inflammatory and antihyperalgesic activity [4] (De Souza et al., 2012).

In order to carry out an effective conservation and domestication strategy for these species as genetic resources and make feasible its economic use in a non-extractive way, knowledge about the genetic variability of *Byrsonima* species it's necessary [2] (Vieira et al., 2010). A highly useful tool that allows the determination of genetic variability and genetic identity is the molecular marker, such as microsatellite markers. SSR markers are widely used for detecting genetic diversity and population structure, gene flow patterns, the incidence of genetic drift, paternity analysis, linkage of phenotypic and genotypic variation, identification of conservation units, and creation of germplasm banks [5] (Semagn et al., 2006). These markers are potentially transferable among related taxa, due to the homologous nature of the DNA sequence in the flanking regions of the microsatellites. As these regions are highly conserved between taxa, this allows cross-amplification between related species to be more cost-effective [6] (Barbara et al., 2007).

Aiming at the importance of developing future strategies of conservation and economic exploitation of the numerous species of the genus *Byrsonima* sp. (murici), in the present study we reported the ability of SSR markers developed for *Byrsonima cydoniifolia* [7] (Bernardes et al., 2014) to amplify SSR loci in other seven *Byrsonima* species. Moreover, we characterized the genetic variability of the microsatellite regions transferred for each species, in order to determine how informative are the transferred loci. These polymorphic markers can be used to conduct effective conservation strategy, domestication planning, parentage studies, and creation of germplasm bank using *Byrsonima* sp. as a genetic resource, and moreover to unveil phylogenetic problems for its related taxa.

Materials And Methods

Plant materials and DNA extraction

Leaf material was collected for the cross-amplification test of 7 taxa of genus *Byrsonima*. For the species *Byrsonima intermedia* A. Juss., *Byrsonima verbascifolia* Rich, Ex. Juss, *Byrsonima laxiflora* Griseb, *Byrsonima subterranea* Brade and Markgr, *Byrsonima umbellata* Mart. Ex A.Juss., *Byrsonima linearifolia* A. Juss. from 16 individuals and for the species *Byrsonima viminifolia* A. Juss. from 14 individuals.

The species tested have different localities in the biome Cerrado, Brazil. Individuals of *B. intermedia* encompass the municipality of Sacramento, at Minas Gerais State (rural area corridor); individuals of *B. verbascifolia* and *B. laxiflora* were from the municipality Barra do Garças, at Mato Grosso State (W 52°35'39", S 15°33'18,5" and W 52° 38'36,5", S 15° 42'28,5", respectively); individuals of *B. subterranea* from the municipality of Niquelândia, individuals of *B. umbellata*, *B. linearifolia* and *B. viminifolia* from the municipality of Alto Paraíso de Goiás, both municipalities at Goiás State.

Genomic DNA was extracted from leaves tissues of each individual of *Byrsonima* sp. following the cetyltrimethylammonium bromide (CTAB) 2% protocol [8] (Doyle and Doyle, 1987). DNA yield and quality were visually assessed on 1 % agarose gels.

Cross-species amplification of SSR markers and genotyping

All seventeen primer pairs developed to amplify microsatellite regions for the species *Byrsonima cydoniifolia* [7] (as described in Bernardes et al., 2014) were used to perform the cross-amplification tests on the species of *Byrsonima*. DNA amplification using each microsatellite primer pair was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). The PCR reactions were performed in a total of 10 µl reaction volume: 0.23 mM of primer (forward + reverse), 0.23 µM of dNTP, 3.25 mg of bovine serum albumin (25 mg/ml), and 1× reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂), 0.75 U of *Taq* DNA polymerase (5U - Phoneutria, Belo Horizonte, Brazil), and using 3.75 ng of template DNA. The PCR program performed the amplification following the protocol: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94°C for 1 min, primers specific annealing temperatures (50– 62°C) depending on primers for 1 min, and 72°C for 45 min for a final extension.

The PCR products were submitted to electrophoresis on a 3% agarose gel stained with ethidium bromide (10mg / mL), in the presence of 1X TBE buffer, for 60 minutes at a voltage of 70V, and with a molecular weight marker 100bp. PCR was repeated for microsatellite loci with amplification products, optimizing the minimum annealing temperature of the primer pairs at 48 ° C.

Locus with the cross-amplification pattern was analyzed by capillary electrophoresis. Genotype information was obtained by fluorescence detection based on the separation of fluorophores labeled microsatellite fragments organized in multiplex panels by capillary electrophoresis using an automatic DNA analyzer, ABI-3500 automated sequencer (Applied Biosystems, Foster City, California, USA) and the ROX internal size standard GS500. Forward primers were pre-labeled with fluorophores at their 5' end, having one of the fluorophores described below: green (HEX), blue (6-FAM), and yellow (NED). PCR products were pooled in multiplex panels created according to amplified fragment sizes, could be the same dyes as long as the alleles presented different sizes of at least 10bp, and different dyes to minimize costs and maximize efficiency. Allele binning and calling was performed using GENEMAPPER 5.0 software (Applied Biosystems), using a detection window (bin) of 1.99 bp.

Validation of transferred SSR markers and genetic diversity analysis

The individual discrimination power was calculated through the genetic identity index (I) [9] (Paetkau, 1995); and the paternity exclusion index (Q) (Weir, 1996) using the IDENTITY 1.0 program [10] (Wagner and Sefc, 1999) from the allelic frequencies for each locus and for the total set of loci. All microsatellite loci transferred were evaluated for their use as polymorphic markers for population genetics studies, so analysis of genetic diversity, i.e., the number of alleles per locus (*A*), observed heterozygosity (*H_o*), and expected heterozygosity (*H_e*) were performed by the program FSTAT 2.9.3.2 [11] (Goudet, 2002). Also, tests of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (*P* > 0.05) in all pairs of loci, in both, using Bonferroni correction.

For each locus the values of the effective number of alleles per locus (A_E) were measured, calculated from the formula: $A_E = 1/(1-H_E)$, and the values of maximum possible heterozygosity, based on the number of alleles per locus (A), from the formula: $H_E \text{ máx} = (A-1)/A$. The proportion of the maximum genetic diversity obtained by the transferred markers was calculated by $H_E / H_E \text{ máx}$ (%) [12] (Moe et al., 2012).

Results

Cross-species amplification of SSR markers

In general, we obtained success in the cross-amplification of seventeen SSR markers developed for *Byrsonima cydoniifolia* A. Juss in the other seven related taxa. Data with the name of locus, primer sequence and number of Genbank accession are available in Supplementary information. For the 17 pairs of tested primers, the presence of satisfactory amplification, i.e. polymorphic loci without nonspecificity, ranged from 11 loci (64.8%) in *B. viminifolia* to 6 loci (35.2%) in *B. umbellata*. Only the species *B. laxiflora* presented two monomorphic loci (11.8%). The presence of non-specific amplification was found for almost all species, with the exception of *B. umbellata* and *B. viminifolia*, ranging from three loci (17.7%) in *B. intermedia* to one locus (5.8%) in *B. subterranea* and *B. linearifolia* (Table 1).

Table 1

Results of cross amplifications in absolute numbers of microsatellite loci for the species *B. intermedia*, *B. verbascifolia*, *B. laxiflora*, *B. umbellata*, *B. subterranea*, *B. viminifolia* e *B. linearifolia*.

Species	Polymorphic loci	Monomorphic loci	Non-specific amplification	No amplification	Total
<i>B. viminifolia</i>	11	0	0	6	17
<i>B. subterranea</i>	9	0	1	7	17
<i>B. intermedia</i>	9	0	3	5	17
<i>B. laxiflora</i>	9	2	2	4	17
<i>B. verbascifolia</i>	8	0	2	7	17
<i>B. linearifolia</i>	7	0	1	9	17
<i>B. umbellata</i>	6	0	0	11	17

For cross-amplification parameters, the annealing temperature ranged from a minimum of 50°C to a maximum of 62°C, while the allele size range (bp) varied from a maximum amplitude of 136–216 bp to a minimum amplitude of 137–139 bp for the BCY16 locus in *B. umbellata*. No linkage disequilibrium were detected for any pair of loci for the seven species. After Bonferroni correction, significant deviations from the Hardy–Weinberg equilibrium ($p < 0.05$) for the fixation index (F_{IS}) were observed for four locus (Table 2).

Table 2

Cross-amplification parameters of microsatellite markers developed for *B. cydoniifolia* in seven closely related species.

Locus	<i>B. viminifolia</i>		<i>B. subterranea</i>		<i>B. intermedia</i>		<i>B. laxiflora</i>		<i>B. verbascifolia</i>		<i>B. linearifolia</i>		<i>B. umbellata</i>	
	Ta (°C)	Allele size range (bp)	Ta (°C)	Allele size range (bp)	Ta (°C)	Allele size range (bp)	Ta (°C)	Allele size range (bp)	Ta (°C)	Allele size range (bp)	Ta (°C)	Allele size range (bp)	Ta (°C)	Allele size range (bp)
BCY01	50*	176–208	-	-	-	-	-	-	-	-	-	-	-	-
BCY02	52	118–178	52*	137–185	59*	100–140	-	-	-	-	50	130–184	52	108–118
BCY03	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BCY04	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BCY05	52*	177–189	52*	177–203	54*	168–216	62	178–190	52	177–193	50*	171–195	-	-
BCY06	52	165–175	54	159–179	58	158–182	54	167–175	58	165–195	-	-	-	-
BCY07	53	183–211	-	-	58	184–206	60	184–194	-	-	50	185–205	-	-
BCY08	54	151–179	52	149–195	52	140–184	50	155–161	52	151–181	50	161–205	-	-
BCY09	55*	149–183	52	168–200	58	164–230	60	171–191	56	165–179	50	149–221	52*	150–152
BCY10	55	177–187	52	177–191	50	168–196	50	173–181	52	173–189	50	169–185	52	175–181
BCY11	53	135–183	-	-	-	-	50	141–179	-	-	-	-	-	-
BCY12	55	194–208	54	182–204	58	192–210	54	194–202	62	194–202	50	182–208	52	190–200
BCY13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BCY14	-	-	52	170–196	59	136–216	58	162–180	52	170–180	-	-	52	161–175
BCY15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BCY16	54	136–146	52	136–148	-	-	-	-	56	138–148	-	-	52	137–139
BCY17	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ta = optimized annealing temperature; * Loci with significant deviations from HWE.

Deviations from HWE occurred due to the sampling effect, in this case, only 16 individuals or 14 individuals per species, since the number of genotyped individuals may not be sufficient to find individuals in all possible genotypic classes, causing deviations in genotypic frequencies. For a locus to be HWE compliant, it must meet many conditions, such as conformance with Mendelian segregation, random mating, absence of recent mutations and genetic drift, and also infinite population. thus, deviation from the HWE test can indicate that one of the conditions is not being met, in this case, infinite population, since it is usually required a large sample to conform to the 'infinity population' requirement [13] (Salanti et al., 2005).

Genetic diversity analysis with SSR transferred markers

B. viminifolia obtained an average of 7,091 alleles/locus, ranging from 3 alleles (BCY16) to 11 (BCY08) allele. *B. subterranea* showed an average of 7.77 alleles/locus, ranging from 4 alleles (BCY16) to 12 (BCY02) alleles. *B. intermedia* identified an average equal to 5.556 alleles/locus, ranging from 3 (BCY12) to 11 (BCY02) alleles. *B. laxiflora* showed an average equal to 5,889 alleles/locus, varying from 3 (BCY12) to 10 (BCY09) alleles. In *B. verbascifolia* it was possible to verify an average of 5.875 alleles/locus, varying from 4 alleles (BCY12 and BCY09) to 7 (BCY06 and BCY14) alleles. *B. linearifolia* showed an average equal to 4,857 alleles/locus, ranging from 2 (BCY10) to 10 (BCY02) alleles. *B. umbellata* presented an average of 2.83 alleles/locus, ranging from 2 alleles (BCY09, BCY12 and BCY16) to 4 alleles (BCY06 and BCY14) (Table 3).

Table 3

Characterization and comparison of the genetic variability of the loci transferred to the species *B. viminifolia*, *B. subterranea*, *B. intermedia*, *B. laxiflora*, *B. verbascifolia*, *B. linearifolia*, and *B. umbellata*

Species	N	SSR locus	A	H _E	H _O	Q	I	H _E /H _E máx (%)
<i>B. viminifolia</i>	14	11	7.091	0.774	0.567	0.999	4.68 x 10 ⁻¹²	92
<i>B. subterranea</i>	16	9	7.770	0.778	0.687	0.999	5.2 x 10 ⁻¹¹	91
<i>B. intermedia</i>	16	9	5.556	0.626	0.507	0.999	1.37 x 10 ⁻¹⁰	78
<i>B. laxiflora</i>	16	9	5.889	0.574	0.503	0.989	5.41 x 10 ⁻⁷	71
<i>B. verbascifolia</i>	16	8	5.875	0.706	0.641	0.993	1.27 x 10 ⁻⁷	86
<i>B. linearifolia</i>	16	7	4.857	0.580	0.447	0.976	9.91x10 ⁻⁶	77
<i>B. umbellata</i>	16	6	2.830	0.436	0.312	0.778	2.5 x 10 ⁻³	69

N = number of individuals; SSR locus = number of microsatellite transferred markers; A = mean number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; Q = paternity exclusion index ; I = genetic identity index ; H_E/H_E máx (%) = proportion of the maximum genetic diversity obtained by the transferred markers.

The detected of maximum possible genetic diversity using transferred SSR markers ranged from 92% (*B. viminifolia*) to 69% (*B. umbellata*). The observed heterozygosity (H_O) values ranged from 0.687 (*B. subterranea*) to 0.312 (*B. umbellata*). The values of expected heterozygosis (H_E) ranged from 0.778 (*B. subterranea*) to 0.436 (*B. umbellata*).

The highest values of genetic diversity were found for *B. subterranea* (H_E = 0,778) using 9 transferred SSR markers and *B. viminifolia* (H_E = 0,774) using 11 transferred SSR markers (Table 3). Therefore, it is noted that despite having a greater number of markers for *B. viminifolia*, this fact did not demonstrate an increase in the genetic diversity values compared to other species with smaller amounts of 8 or 9 SSR markers.

Greater genetic diversity were denoted in *B. verbascifolia* (H_E=0.706; H_O=0.641) individuals, using eight SSR markers transferred, than in *B. intermedia* (H_E=0.626; H_O=0.507) and *B. laxiflora* (H_E=0.574; H_O=0.503) individuals, both using 9 SSR markers transferred.

Although the number of SSR markers transferred for each species varies, an average of 8.43 markers per species, it is noted that when transferred a minimum of 7 markers (*B. linearifolia*), they proved to be a set of highly informative markers for such species, able to exclude false paternity and demonstrated efficiency in discriminating individuals.

The lowest values of H_E and H_O were identified in *B. umbellata* ($H_E=0.436$; $H_O=0.312$), probably due to the small number of SSR markers transferred (6). The set of developed SSR markers presented reasonable information ($I=0.778$; $Q=2.5 \times 10^{-3}$). Thus, it is necessary to develop more SSR markers to detect high levels of genetic variability for future advanced studies in population-genetics for this species.

Discussion

One of the limitations of microsatellite markers is their obtaining, since it is necessary to have prior knowledge of the DNA sequence, to develop specific primer pairs flanking each identified microsatellite region [14] (Vieira et al., 2016). However, an alternative methodology is transferability, since microsatellite markers are potentially transferable to phylogenetically close species, due to the homologous nature of the DNA sequence in the flanking regions of the microsatellites, reducing the costs and long time to develop of this kind of markers [6] (Barbara et al., 2007).

In this work, microsatellite markers were obtained for all the seven *Byrsonima* species tested through transferability. Highlighting that of these seven Brazil native species, four are endemic species (*B. intermedia*, *B. laxiflora*, *B. umbellata* and *B. viminifolia*). The transferability of SSR markers developed for *B. cydoniifolia* in the species *B. intermedia*, *B. laxiflora*, *B. verbascifolia*, *B. umbellata*, *B. subterranea*, *B. viminifolia* and *B. linearifolia* showed high efficiency, varying from 11 loci transferred (64.8%) in *B. viminifolia* to 6 transferred loci (35.2%) in *B. umbellata*.

In Brazil, studies related to the development and use of microsatellite markers in plants are approximately two decades old and were first used in studies of forest species such as *Ceiba pentandra* [15] (Brondani et al., 2003), *Araucaria angustifolia* [16] (Schmidt et al., 2007), and cultivated *Oryza glumaepatula* [17] (de Campos Vaz et al., 2009). Several studies with microsatellite markers are also been developed for native Cerrado species *Hymenaea courbaril* [18] (Ciampi et al., 2008), *Tibouchina papyrus* [19] (Telles et al., 2011), *Eugenia dysenterica* [20] (Telles et al., 2013), *Byrsonima cydoniifolia* [7] (Bernardes et al., 2014), *Dipteryx alata* [21] (Guimarães et al., 2019) *Hymenaea stigonocarpa* [22] (Gonçalves et al., 2019). Thus, this work increases the number of microsatellite markers developed for Cerrado biome species.

Similar results was found for another *Byrsonima* species by [23] Croft and Schaal (2012). Eight SSR markers were developed for the species *Byrsonima crassifolia* showing an average of 5.4 alleles/locus in four populations, ranging from two to 11 alleles. The heterozygosity observed at the loci among the four tested populations of *B. crassifolia* ranged from 0.000 to 0.933, while the genetic diversity ranged from 0.000 to 0.839. Here, considering the seven species, it is verified that the observed heterozygosity of the loci varied from the minimum value of 0.000 (*B. umbellata* – locus 09) to the maximum value of 1,000 (*B. laxiflora* – locus 09), while the genetic diversity varied from 0.063 (*B. intermedia*) to 0.924 (*B. viminifolia*).

In another study, they tested the transferability of ten markers developed for *B. crassifolia* in three other species of *Byrsonima* sp. The ten tested loci were transferred to *B. pachyphylla* ($A=3.6$; $H_E=0.481$; $H_O=0.322$) and *B. verbascifolia* ($A=5.2$; $H_E=0.604$; $H_O=0.477$), while only seven loci were transferred to *B. coccolobifolia* ($A=4.6$; $H_E=0.458$; $H_O=0.337$) [24] (Menezes et al., 2014). In this study, the set of eight SSR loci developed to *B. verbascifolia* was capable to detect higher values of genetic variability ($A=5.875$ $H_E=0.706$; $H_O=0.641$) than values founded by [24] Menezes et al.. (2014) from the ten loci transferred from *B. crassifolia* to *B. verbascifolia*.

Thus, it is concluded that microsatellite marker panels transferred to the species *B. intermedia*, *B. verbascifolia*, *B. laxiflora*, *B. subterranea*, *B. viminifolia* and *B. linearifolia* are very informative, with a high combined probability of exclusion of paternity ($Q \geq 0.976$) and the low combined probability of identity ($I \leq 9.91 \times 10^{-6}$), so these markers are potentially suitable for future genetic-population studies. But, the set of markers transferred to *B. umbellata* is reasonably informative ($Q=0.778$; $I=2.5 \times 10^{-3}$), so it is necessary to develop more microsatellite markers for genetic-population studies for this species.

Considering phylogenetic studies, it is known that the genus *Byrsonima* is a monophyletic group within Malpighiaceae, confirmed by molecular and morphological synapomorphies. The genus *Byrsonima* is traditionally divided into two

subgenera (*Byrsonima* subg. *Byrsonima* and *Byrsonima* subg. *Macrozeugma* Nied), initially proposed based on the stamen morphology [25] (Francener, 2016).

According to [25] Francener 2016, the subgenera can be separated based on the color of the petals, in addition to the morphology of the anther. In the subgenus *B.* subg. *Byrsonima* the posterior petal is yellow, and the connective does not surpass the anther locules or surpass only one-quarter of its size. While in *B.* subg. *Macrozeugma* the posterior petal is white or pink and the connective surpasses the locules in more than one-quarter of its size.

Among the species of *Byrsonima* sp. in this study, regarding the morphological characteristics, the species *B. viminifolia*, *B. subterranea*, *B. intermedia*, *B. laxiflora*, *B. linearifolia* presents terminal inflorescences with yellow colored petals and are included in the subgenera *Byrsonima* subg. *Byrsonima*, as also the specie *B. cydoniifolia*. While, *B. umbellata* is the only species, in this study, with white petals in the terminal inflorescences and belongs to the subgenera *B.* subg. *Macrozeugma*.

Therefore, the reason for the low number of microsatellites markers transferred to *B. umbellata* (six markers) compared to the other species (above 7 to 11 markers), is due to the lower efficiency in cross-amplification, probably because of the greater evolutionary distance of *B. umbellata* with *B. cydoniifolia*, being included in different subgenera. Once that the rate of successful amplification decreases as the genetic divergence between species increases [6] (Barbara et al., 2007).

According to [26] Mamede et al. (2018) when analyzing two species of *Byrsonima* belonging to different groups: *Byrsonima coccolobifolia* (*Byrsonima* subg. *Macrozeugma*) known as "murici-rosa". and *Byrsonima crassifolia* (L.) Kunth (*Byrsonima* subg. *Byrsonima*) commonly called "murici-Amarelo", there was a set of 20 regions with high divergence, most of them intergenic sequences. Evidencing the genetic divergence between *Byrsonima* species belonging to the different subgenera.

Finally, the transferability technique for the development of microsatellite markers between different species of *Byrsonima* is effective and capable of generating numerous sets of microsatellite markers. This fact is fundamental and relevant for species of genus *Byrsonima*, Given the scope of this complex of species in Brazil, for which microsatellite markers have not yet been provided.

So, the markers developed in this study for *Byrsonima* species by transferability can provide relevant information in advanced studies in population-genetics for these species, once they are capable of detecting different magnitudes of genetic variability within and between populations, so can be used for identification of conservation units and for the investigation of genetic processes that occur in populations, such as the genetic diversity of populations, patterns of gene flow, the incidence of genetic drift and generation of the genetic neighborhood. So, different strategies for in situ or ex-situ conservation can be admitted and also for these species domestication, as a genetic resource.

Declarations

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Conflict of Interest

The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animal.

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

D.M. Murakami, N. Bizão, M. J. SILVA; Marcos José provided the plant material. M.P.C. Telle, D.M. Murakam and N. Bizão conceived the idea and provide necessary resources. V. Bernardes and T.N. Souza designed the experiments, mined the data and perform the experiments. V. Bernardes and M.P.C. Telles wrote the manuscript. All authors have read and approved the final manuscript.

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