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Cross-amplification and characterization of microsatellite markers in species of *Manihot* Mill. (Euphorbiaceae) endemic to the Brazilian Cerrado

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ABSTRACT

The genus *Manihot* Mill. contains about 120 species of which about 104 occur in Brazil. We tested the cross-amplification of ten microsatellite markers developed for *Manihot esculenta* in 15 species of *Manihot* endemic to the Brazilian Cerrado. We also evaluated the genetic diversity of *Manihot irwinii*, *M. orbicularis*, and *M. purpureocostata*. Ten pairs of primers were amplified among 14 species of *Manihot*. The percentage of polymorphic loci per species varied from 70 to 100 %. Nine markers showed amplification and polymorphism when evaluated on polyacrylamide gel. The markers were combined to form three sets for multiplex genotyping for genetic diversity analysis, and showed 51, 75, and 75 alleles in *M. irwinii*, *M. orbicularis*, and *M. purpureocostata*, respectively. The levels of genetic diversity for the transferred markers were high for the three species and proved to be useful for population genetics studies of species of *Manihot* endemic to the Cerrado. The results of this study will help to better understand the genetic diversity, taxonomy and relationships among species *Manihot*, and to develop conservation programs for the genus.

Keywords: Genetic diversity, polymorphism, SSR, transferability wild cassava

Introduction

The genus *Manihot* has a Mesoamerican origin with its center of diversity in Brazil (Silva 2014). The Cerrado of Central Brazil presents about 104 documented species, so the main center of diversity for the genus within the country, (Silva & Amaral 2020).

Floristic and taxonomic studies have shown that species of this genus have problems of taxonomic delimitation due to the lack of taxonomic studies in Brazil. Nonetheless, several new species have been described in recent years (Silva *et al*. 2016; Silva 2016; Silva *et al*. 2017; Mendoza *et al*. 2018), showing significant advances in knowledge of the diversity of the genus. However, little is known about the genetic diversity of these species, especially in the Central-West Region of Brazil.

The development of microsatellites or simple sequence repeats (SSRs) provides an ideal tool for investigating patterns of genetic variation due to their codominant

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inheritance and multiallelic and highly polymorphic properties, as well as being abundant and well distributed throughout the genome (Li *et al*. 2002; Ellegren 2004). However, given the time-consuming and expensive process of isolating SSRs, it is advantageous to test available microsatellite markers for phylogenetically close species by cross-amplification before investing in the development of species-specific markers.

The success of cross-species amplification depends on the conservation of primer sites within flanking sequences and on the maintenance of sequences that promote polymorphisms (FitzSimmons *et al*. 1995). Several studies have demonstrated the utility of using primer pairs designed from one species for others of the same genus (Bernardes *et al*. 2014; Buzatti *et al*. 2016) and even for species of other genera (Barbará *et al*. 2007; Santos *et al*. 2015; Fagundes *et al*. 2016; Miranda *et al*. 2016).

Considering the insufficient knowledge available regarding the diversity of wild species of the genus *Manihot*, along with the taxonomic and phylogenetic complexity of the genus and the lack of SSR and molecular information, we tested microsatellite markers developed for *M. esculenta* (cultivated cassava) (Chavarriaga-Aguirre *et al*. 1998; Mba *et al*. 2001) by cross-amplification in 15 congeners endemic to the Cerrado. Moreover, we evaluated the genetic diversity of the markers in three of these species (*M. irwinii*, *M. orbicularis*, and *M. purpureocostata*), to provide tools for population genetics studies on species of *Manihot* endemic to the Cerrado.

Materials and methods

Plant material and cross-species amplification

Amplification tests used leaves sampled from three individuals from each of the know 15 species of *Manihot* of the Cerrado, Goiás State, Central-West Brazil (Tab. 1). Marker polymorphism evaluation used eight individuals per species. A standard protocol of 2 % CTAB was used for DNA extraction (Doyle & Doyle 1987). Ten nuclear microsatellite markers previously developed for *M. esculenta* were tested in the 15 *Manihot* species: GA 134, GA 136, AG 126, AG 21, AG 12, AG 131, AG 16, GAGG 5 (Chavarriaga-Aguirre *et al*. 1998), and SSRY 12 and SSRY 82 (Mba *et al*. 2001).

PCR amplifications were performed in a final volume of 10 µl using 4.5 ng template DNA, 0.18 µM primers (forward + reverse), 0.15 µM dNTP, 2.16 mg bovine serum albumin (BSA), 1x reaction buffer (10 mM Tris), HCl [pH 8.3], 50 mM KCl, 1.5 mM MgCl 2, and 0.75 units of Taq DNA polymerase (5U; Phoneutria, Belo Horizonte, Brazil) under the following conditions: 30 cycles of 94 °C for 1 min, 54–65 °C (depending on the initiator, Tab. 2) for 1 min, and of 72° C for 1 min, and a final extension at 72 °C for 45 min.

Table 1. Sampling locations for the *Manihot* species used in the present study and the vouchers deposited in the herbarium of the Universidade Federal de Goiás.

Species	Population	Geographical coordinates		Altitude (m)	Voucher/ Herbarium Universidade Federal de Goiás	References used to identify each species
		Longitude	Latitude			
M. gabrielensis Allem	Água Fria de Goiás- Goiás	-47.6045	-14.9874	1131	M. J. Silva 4032 (UFG)	Allem (1989)
M. violacea Pohl	Pirenópolis - Goiás	-48.9172	-15.8394	925	M. J. Silva 5321(UFG)	Pohl (1827)
M. saxatilis M. J. Silva & Sodré	Alto Paraíso de Goiás - Goiás	-47.7369	-14.1113	1155	M. J. Silva 4702 (UFG)	Silva & Sodré (2014)
M. alutacea D. J. Rogers & Appan	Serra do Pouso Alto - Goiás	-47.5081	-14.0541	1083	M. J. Silva 5522 (UFG)	Rogers & Appan (1973)
M. attenuata Müll. Arg.	Alto Paraíso de Goiás - Goiás	-47.7931	-14.1453	1030	M. J. Silva 4503 (UFG)	Muller (1874)
M. divergens Pohl	Água Fria de Goiás- Goiás	-47.6045	-14.9874	1131	M. J. Silva 4028 (UFG)	Pohl (1827)
M. mossamedensis Taub.	Parque Estadual Serra Dourada - Goiás	-50.1802	-16.0671	997	M. J. Silva 3527 (UFG)	Taubert (1896)
M. confertiflora M. J. Silva	Alto Paraíso de Goiás - Goiás	-47.6326	-14.1679	1146	M. J. Silva 5572 (UFG)	Silva (2015)
M. pentaphylla Pohl	Pirenópolis - Goiás	-48.9172	-15.8394	925	M. J. Silva 6342 (UFG)	Pohl (1827)
M. paviifolia Pohl	Mossâmedes- Goiás	-50.8579	-16.0746	985	M. J. Silva 3126 (UFG)	Pohl (1827)
M. peltata Pohl	Santa Bárbara - Goiás	-47.750	-16.9833	638	M. J. Silva 4825(UFG)	Pohl (1827)
M. tripartita (Spreng) Müll. Arg.	Cocalzinho de Goiás- Goiás	-48.8333	-15.7819	1157	M. J. Silva 5821 (UFG)	Muller (1866)
M. orbicularis Pohl	Alto Paraíso de Goiás- Goiás Colinas do Sul - Goiás	-47.4232 -47.9668	-14.1735 -14.1668	1028 649	M. J. Silva 4384UFG) M. J. Silva 5504 (UFG)	Pohl (1827)
	Pirenópolis - Goiás	-49.0376	-15.7423	808	M. J. Silva 6253 (UFG)	
M. purpureocostata Pohl	Alto Paraíso de Goiás - Goiás Alto Paraíso de Goiás/Vale da Lua - Goiás	-47.7913 -47.7801	-14.1599 -14.1749	1072 1020	M. J. Silva 4531 (UFG) M. J. Silva 3867 (UFG)	Pohl (1827)
	Alto Paraíso de Goiás/Vila São Jorge - Goiás	-47.8239	-14.1749	982	M. J. Silva 4150 (UFG)	
M. irwinii D. J. Rogers & Appan	Corumbá de Goiás - Goiás Pirenópolis - Goiás	-48.7853 -49.0458	-15.8403 -15.7208	1067 730	M. J. Silva 5805 (UFG) M. J. Silva 6389 (UFG)	
	Cocalzinho - Goiás	-48.8333	-15.7819	1157	M. J. Silva 6406 (UFG)	

Table 2. Cross amplification of ten microsatellite loci of *Manihot esculenta* Crantz for 15 wild species of *Manihot* endemic to the Cerrado.

Species	Locus / Annealing temperature (°C)										Successful amplification of *Manihot* primers	Polymorphic *Manihot* SSR markers
	GA 21	GA 126	GA 136	GA 134	GAGG5	GA12	GA16	GA131	SSRY82	SSRY12	Number	Number
M. gabrielensis	58	63	61	58	54	61	64	60	58	61	10	10
M. violacea	58	60	58	58	54	58	62	60	58	61	10	10
M. orbicularis	60	60	58	56	56	58	60	56	62	62	10	10
M. purpureocostata	60	60	58	56	56	58	60	56	62	62	10	10
M. saxatilis	58	60	59	58	55	60	65	61	59	63	10	10
M. alutacea	58	58	59	58	55	62	65	61	59	63	10	10
M. attenuata	58	58	60	59	54	60	65	61	59	61	10	10
M. divergens	58	60	59	58	55	60	61	58	57	61	10	10
M. irwinii	56	56	56	56	-	56	56	56	56	56	9	9
M. mossamedensis	56*	60	58	56	65	58	64	56	58	62	10	9
M. confertiflora	60	58*	58	59	54	60	64	58	59	61	10	9
M. pentaphylla	62	58	54	56	56	58	58*	56	58	60	10	9
M. paviifolia	61*	58*	56	56	58	62	60	61	58	63	10	8
M. peltata	60	63	60	58	60*	60*	60	61	58*	61	10	7
M. tripartita	59	60*	58	56	56	58	60*	56	58*	60	10	7
Number of species with polymorphism	13	12	15	15	13	14	13	15	13	15		

* Monomorphic locus: did not amplify.

The annealing temperature of the primers was adjusted for each marker until an acceptable amplification pattern was found on 3 % agarose gel (Tab. 2). Polymorphism of the markers in the 15 species of *Manihot* was determined through standard 6 % acrylamide gel electrophoresis visualized by silver staining procedures (Creste *et al*. 2001). Allele size was determined by reference to 10 bp and 50 bp DNA standards (Invitrogen ™).

Genetic variability of polymorphic loci

The markers that showed the best polyacrylamide gel amplification profiles, associated with verified polymorphism in the 15 species, were selected for characterizing the genetic diversity of three *Manihot* species: *M. orbicularis* Pohl, *M. purpureocostata* Pohl, and *M. irwinii* DJ Rogers & Appan. A sample of 24 individuals, representing known occurrence, was used for each species, for a total of 72 individuals. DNA extraction and amplification followed the same protocols as described above.

Forward sequences of selected primer pairs were labeled with one of four fluorescent dyes: VIC, NED, 6-FAM, or PET. The sizes of amplification products were determined using a GeneScan 600 LIZ internal marker (Applied Biosystems) in an ABI PRISM® 3500 DNA Genetic Analyzer (Applied Biosystems). Microsatellite loci with greater clarity in their amplification detected by capillary electrophoresis were arranged in multiplex panels for analysis of the three *Manihot* species.

Allele calling was performed using GeneMapper 5.0 software (Applied Biosystems). Genotypes were confirmed using an allelic ladder constructed with all alleles found for each locus in this study. Micro-Checker software (Oosterhout *et al*. 2004) was then used to detect errors due to stuttering, allele dropout, and null alleles.

The power of individual discrimination with the total loci set and with each locus was evaluated by estimates of probability of genetic identity (I) (Paetkau *et al*. 1995) and the probability of paternity exclusion (Q) (Weir 1996), using Identity 1.0 software (Wagner & Sefc 1999). Genetic variability analysis, including allelic richness, observed heterozygosity (H_o), and expected heterozygosity under the Hardy-Weinberg equilibrium (H_e), were estimated using Genetic Data Analysis 1.0 software (GDA) (Lewis & Zaykin 2001). Linkage disequilibrium was evaluated using Bonferroni correction in FSTAT 2.9.3.2 software (Goudet 2002).

Results and discussion

The markers amplified in all 15 species of *Manihot* were dinucleotides, most with the GA motif. The least conserved marker was GAGG5 (tetranucleotide), which did not amplify for one of the 15 species (*M. irwinii*).

All ten primer pairs tested in the wild *Manihot* species were polymorphic (100 %) in nine of them (*M. gabrielensis, M. violacea, M. orbicularis, M. purpureocostata, M. saxatilis, M. alutacea, M. attenuata, M. divergens,* and *M. irwinii*), while nine (90 %) were polymorphic for *M. mossamedensis, M. confertiflora,* and *M. pentaphylla*; eight (80 %) for *M. paviifolia*; and seven (70 %) for *M. peltata* and *M. tripartita* (Tab. 2). The results obtained regarding the percentage of polymorphic loci among the three species of *Manihot* are similar to those reported in the literature for other species of the same genus and characterized by a set of similar primers. Among these, we have included the cultivated

species *M. esculenta* and six other different species (all wild), *M. esculenta subsp. flabellifolia, M. esculenta subsp. peruviana, M. aesculifolia, M. brachyloba, M. carthaginensis*, and *M. tristis* (Roa *et al*. 2000). Thus, in the present study, the percentage of polymorphic loci obtained from cross-amplification among species within the genus *Manihot* tends to maintain close to 100 %, reflecting high conservation of genomic regions among species. This conservation may be evidence of recent diversification of the genus *Manihot*, which would explain the low taxonomic resolution for some sets of *Manihot* species (Duputié *et al*. 2011).

The high microsatellite cross-amplification observed among species of *Manihot* reveal their genetic proximity with *M. esculenta*. This finding corroborates the results of Raji *et al*. (2009), who showed that 92 % of the markers were transferable to wild relatives of *M. esculenta* belonging to the same genus (*M. epruinosa, M. glaziovii, M. brachyandra,* and *M. tripartita*), while only a small fraction was transferable to species of other genera.

Cross-species amplification has been reported for six wild species of the genus *Manihot* (*M. aesculifolia, M. brachyloba, M. carthaginensis, M. esculenta* subsp. *flabellifolia, M. esculenta* subsp. *peruviana,* and *M. tristis*) (Roa *et al*. 2000), showing that, in general, microsatellite primers work throughout the genus. However, as phylogenetic distance increases, successful amplification of loci tends to decrease. This relationship with phylogenetic distance was also observed by Bressan *et al*. (2012) when reporting cross-amplification with the species *Jatropha curca*, which belongs to the same family as *Manihot* (Euphorbiaceae). They found amplification of alleles in species of the same genus, but not in other genera of Euphorbiaceae, such as *Hevea brasiliensis, Manihot esculenta,* and *Ricinus communis*. Successful transferability of microsatellite markers among closely related species has also been verified for other Cerrado species, such as *Anacardium humile* (Soares *et al*. 2013), *Byrsonima cydoniifolia* (Bernardes *et al*. 2014), and *Campomanesia adamantium* and *C. pubescens* (Miranda *et al*. 2016). This success can be explained by the conservation of microsatellite flanking regions in closely related species (FitzSimmons *et al*. 1995; Barbará *et al*. 2007).

The markers were polymorphic for most species (eight individuals), varying among 12 to 15 species. The most polymorphic markers were GA 136, GA 134, GA131, and SSRY12, which were polymorphic in all 15 species (Tab. 2). Such high levels of polymorphism were expected according to data in the literature for wild species of the genus *Manihot* (Roa *et al*. 2000; Raji *et al*. 2009).

Out of the ten markers, nine exhibited better amplification and polymorphism patterns and so were combined into three sets for multiplex genotyping and characterization of the loci (Tab. 3). Out of the nine markers with clear amplicons tested on the three wild species of *Manihot*, four loci had null alleles, the locus GA136 for *M. orbicularis* and *M. irwinii*, GA126 for *M. orbicularis* and *M. purpureocostata*, GA16 for *M. irwinii*, and SSRY12 for *M. orbicularis*. Null alleles have been commonly found in studies of transferability of microsatellite markers in wild species of *Manihot* (Roa *et al*. 2000; Raji *et al*. 2009). However, no null alleles have been observed in *M. esculenta* (Roa *et al*. 2000). In this sense, the the occurrence de null alleles may be an artifact in the sequences flanking the microsatellite, caused by transferability (Dabrowski *et al*. 2015).

No significant changes in linkage disequilibrium ($P > 0.05$) were found for any pair of loci in any species. Deviations from HWE ($P < 0.05$) were observed at locus GA21 for all species, GA136 for *M. irwinii* and *M. orbicularis*, GA16 and SSRY82 for *M. irwinii*, GA126 for *M. orbicularis* and *M. purpureocostata*, and GA131 for *M. orbicularis*. Among the loci that deviated from HWE, some showed null alleles, which may explain the deviation. Analysis with more populations and a larger number of individuals per population may confirm this result.

The present study detected 51 alleles in *M. irwinii*, 75 alleles in *M. orbicularis* and 75 alleles in *M. purpureocostata,* which demonstrates a high level of polymorphism. These results are similar to those reported in the literature for wild species of the genus *Manihot*, which found 79 alleles (Roa *et al*. 2000) and 50 alleles (Silva *et al*. 2017). Studies of *M. esculenta* have found 46 alleles (Siqueira *et al*. 2009), 45 alleles (Roa *et al*. 2000), and 47 alleles (Aragon *et al*. 2012). These results follow Roa *et al*. (2000), who state that the wild species of the genus *Manihot* have a larger pool of SSR alleles than *M. esculenta*.

The number of alleles per locus found for the three species of *Manihot* varied from three to 16, with averages

Table 3. Three sets of microsatellite markers for multiplex genotyping for the species *Manihot irwinii*, *M. orbicularis* and *M. purpureocostata*.

Multiplex	Locos	Fluorescence	Overall amplitude of allele size (bp)	Amplitude of allele size		
				M. irwinii	*M. orbicularis*	*M. purpureocostata*
1	GA 134	6- FAM	303 - 369	309-337	315-341	303-369
	GA 136	PET	154 - 176	156-172	154-176	154-164
	GA 126	NED	202 - 210	204-208	202-210	204-210
	GA 21	VIC	103 - 119	103-107	103-119	103-117
2	GA 12	6-FAM	131 - 173	132-156	131-165	131-173
	GA 131	PET	88 - 99	88-98	91-99	89-99
	GA 16	NED	99 - 111	101-105	99-109	101-111
3	SSRY 12	NED	180 - 294	254-282	180-294	236-294
	SSRY 82	6-FAM	172 - 190	172-190	180-188	172-184

Table 4. Genetic characterization of nine microsatellite loci of *Manihot esculenta* in three wild species of *Manihot*. Species (number of individuals); A: number of alleles per loco; H_e: heterozygosity expected by the Hardy-Weinberg equilibrium; H_o: observed heterozygosity; Q: probability of paternity exclusion; I: probability of identity.

Loco	*M. irwinii* (N=24)					*M. orbicularis* (N=24)					*M. purpureocostata* (N=24)				
	A	H_e	H_o	Q	I	A	H_e	H_o	Q	I	A	H_e	H_o	Q	I
GA 21	3	0.656	0.500	0.349	0.203	8	0.773	0.869	0.544	0.094	7	0.797	0.833	0.571	0.082
GA 126	3	0.551	0.625	0.252	0.305	5	0.689	0.416	0.431	0.153	4	0.406	0.217	0.219	0.389
GA 134	11	0.875	0.791	0.719	0.035	13	0.890	0.833	0.746	0.028	16	0.908	0.875	0.781	0.021
GA 136	7	0.786	0.583	0.577	0.08	9	0.742	0.500	0.516	0.108	5	0.460	0.434	0.251	0.337
GA 12	7	0.609	0.583	0.39	0.192	9	0.621	0.625	0.419	0.173	13	0.850	0.916	0.683	0.044
GA 16	3	0.289	0.083	0.133	0.543	6	0.559	0.458	0.342	0.234	6	0.645	0.565	0.394	0.181
GA 131	6	0.541	0.583	0.336	0.244	5	0.713	0.541	0.433	0.149	6	0.549	0.500	0.340	0.239
SSRY 12	5	0.718	0.476	0.439	0.145	15	0.926	0.791	0.812	0.015	13	0.848	0.818	0.669	0.049
SSRY 82	6	0.535	0.428	0.32	0.257	5	0.599	0.500	3.660	0.207	5	0.736	0.863	0.492	0.118
Average	5.7	0.618	0.517	0.992	$1.672x10^{-7}$	8.3	0.724	0.615	0.999	$9.132x10^{-10}$	8.3	0.689	0.669	0.998	$2.614x10^{-9}$

of 5.7, 8.3, and 8.3 alleles per locus for *M. irwinii*, *M. orbicularis,* and *M. purpureocostata*, respectively (Tab. 4). Thus, the high average number of alleles per locus found in the present study suggests that the set of markers used substantially represents the polymorphism of the loci. This can be confirmed by the high polymorphism along with the low probability of identity (1.672×10^{-7}, 9.13×10^{-10}, and 2.61×10^{-9}) and high power of paternity exclusion (0.999, 0.999, and 0.998), observed for the species *M. irwinii, M. orbicularis,* and *M. purpureocostata*, respectively. These results show that the nine markers are suitable for discriminating individuals at each locus under analysis (Paetkau *et al*. 1995) and demonstrated a high power of paternity exclusion (Weir & Evett 1998), allowing an efficient characterization of the genetic variability existing in populations of wild *Manihot* species (Tab. 4).

The average genetic diversity (H_e) of the markers was high for *M. irwinii* (74 %), *M. orbicularis* (82 %), and *M. purpureocosta* (78 %). These results are equivalent to the maximum genetic diversity expected (0.833, 0.879, and 0.879), according to Hennink & Zeven (1991), considering the number of alleles found per locus.

The average observed heterozygosity (H_o= 0.517, 0.615, and 0.669) was lower than the expected (H_e=0.618, 0.724, and 0.689; Tab. 4) for *M. irwinii*, *M. orbicularis,* and *M. purpureocosta*, respectively. This result suggests that the heterozygote deficiency may be due to several factors, such as inbreeding (Halsey *et al.* 2008), limited sample size, and presence of null alleles, with the latter being a common factor in transferability studies with wild species of *Manihot* (Roa *et al*. 2000; Raji *et al*. 2009), and with other species of the Cerrado (Ciampi *et al*. 2008; Feres *et al*. 2009; Soares *et al*. 2013; Fagundes *et al*. 2016; Miranda *et al*. 2016).

The present study documented ten polymorphic microsatellite markers for the 15 studied species of *Manihot*. Nine of these markers were indicated as having high potential to detect genetic variation in the three analyzed wild species of *Manihot*. Thus, the results of this study are promising and valuable for developing further studies of genetic variability of these species and for studies aiming to properly understand the relationships among the species of the genus *Manihot*, as well as their genetic diversity, taxonomy, and conservation.

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References

Allem AC. 1989. Four new species of *Manihot* (Euphorbiaceae) from Brazil. Revista Brasileira de Biologia 49: 649-662.

Aragon E, Aguilar V, Arguello J. 2012. Diversidad genética en cultivares de *Manihot esculenta* Crantz en Nicarágua determinada mediante microsatélites (SSR). Biotecnologia Vegetal 12: 211-218.

Barbará T, Palma-Silva C, Paggi GM, Bered F, Fay MF, Lexer C. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Molecular Ecology 16: 3759-3767.

Bernardes V, Anjos DE, Gondim SGCA, Murakami DM, Bizão N, Telles MPC. 2014. Isolation and characterization of microsatellite loci in *Byrsonima cydoniifolia* (Malpighiaceae) and cross-amplification in *B. crassifolia*. Applications in Plant Sciences 2: 1400016. doi: 10.3732/apps.1400016

Bressan EDA, Scotton DC, Ferreira RR, *et al.* 2012. Development of microsatellite primers for *Jatropha curcas* (Euphorbiaceae) and transferability to congeners. American Journal of Botany 99: 237-239.

Buzatti RSO, Chicata FSL, Lovato MB. 2016. Transferability of microsatellite markers across six *Dalbergia* (Fabaceae) species and their characterization for *Dalbergia miscolobium*. Biochemical Systematics and Ecology 69: 161-165.

Chavarriaga-Aguirre P, Maya MM, Bonierbale MW, *et al*. 1998. Microsatellites in cassava (*Manihot esculenta* Crantz); discovery, inheritance and variability. Theoretical and Applied Genetics 97: 493-501.

Ciampi AY, Azevedo VCR, Gaiotto FA, Ramos ACS, Lovato MB. 2008. Isolation and characterization of microsatellite loci for *Hymenaea courbaril* and transferability to *Hymenaea stigonocarpa*, two tropical timber species. Molecular Ecology Resources 8: 1074-1077.

Creste S, Neto Tulmann A, Figueira A. 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. Plant Molecular Biology Reporter 19: 299-306.

Dabrowski MJ, Bornelöv S, Kruczyk M, Baltzer N, Komorowski J. 2015. "True" null allele detection in microsatellite loci: A comparison of methods, assessment of difficulties and survey of possible improvements. Molecular Ecology Resources 15: 477-488.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Duputié A, Salick J, Mckey D. 2011. Evolutionary biogeography of *Manihot* (Euphorbiaceae), a rapidly radiating Neotropical genus restricted to dry environments. Journal of Biogeography 38: 1033-1043.

Ellegren H. 2004. Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics 5: 435-45.

Fagundes BS, Silva LF, Giacomin RM, Secco D, Díaz-Cruz JA, Da-Silva PR. 2016. Transferability of microsatellite markers among *Myrtaceae* species and their use to obtain population genetics data to help the conservation of the Brazilian Atlantic Forest. Tropical Conservation Science 9: 408-422.

Feres JM, Martinez MLL, Martinez C, Mestriner MA, Alzate-Marin AL. 2009. Transferability and characterization of nine microsatellite markers for the tropical tree species *Tabebuia roseo-alba*. Molecular Ecology Resources 9: 434-437.

FitzSimmons NN, Moritz C, Moore SS. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Molecular Biology and Evolution 12: 432-40.

Goudet J. 2002. FSTAT: a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). http://www2. unil. ch/popgen/softwares/fstat. htm. 10 Oct. 2020.

Halsey ME, Olsen KM, Taylor NJ, Chavarriaga-Aguirre P. 2008. Reproductive biology of cassava (*Manihot esculenta* Crantz) and isolation of experimental field trials. Crop Science 48: 49-58.

Hennink S, Zeven AC. 1991. The interpretation of Nei and Shannon-Weaver within population variation indices. Euphytica 51: 235-240.

Lewis PO, Zaykin D. 2001. GDA (Genetic Data Analysis): Computer Program for the Analysis of Allelic Data (version 1.1). http://phylogeny.uconn.edu/software/. 10 Oct. 2020.

Li Y-C, Korol AB, Fahima T, Beiles A, Nevo E. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Molecular Ecology 11: 2453-2465.

Mba REC, Stephenson P, Edwards K, *et al*. 2001. Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: Towards an SSR-based molecular genetic map of Cassava. Theoretical and Applied Genetics 102: 21-31.

Mendoza M, Simon MF, Arquelão TKM, Cavalcanti TB. 2018. Novas espécies de *Manihot* (Euphorbiaceae) do Brasil Central. Rodriguésia 69: 915-932.

Miranda EAGC, Boaventura-Novaes CRD, Braga RS, Reis EF, Pinto JFN, Telles MPC. 2016. Validation of EST-derived microsatellite markers for two cerrado-endemic *Campomanesia* (Myrtaceae) species. Genetics and Molecular Research 15: 15017658. doi: 10.4238/gmr.15017658

Muller J. 1866. Euphorbiaceae exceto subordo Euphorbieae. Prodromus 15: 189-1286.

Muller JA. 1874. Euphorbiaceae. In: Martius CFP, Eichler AWM. (eds.) Flora Brasiliensis. Leipzig, E. Fleischer. p. 293-750.

Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538.

Paetkau D, Calvert W, Stirling I, Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polars bears. Molecular Ecology 4: 347-354.

Pohl JE. 1827. *Plantarum brasiliae* icones et descriptiones hactenus ineditae: Iussu et auspiciis Francisci Primi, imperatoris et regis augustissimi. Vindobonae, Vienna, Antonii Strauss, Missouri Botanical Garden.

Raji AA, Anderson J V, Kolade OA, Dixon AG, Ingelbrecht IL. 2009. Gene-based microsatellites for cassava (*Manihot esculenta* Crantz): Prevalence, polymorphisms, and cross-taxa utility. BMC Plant Biology 9: 118. doi: 10.1186/1471-2229-9-118

Roa AC, Chavarriaga-Aguirre P, Duque MC, *et al*. 2000. Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. American Journal of Botany 87: 1647-1655.

Rogers DJ, Appan SG. 1973. Flora Neotropica: *Manihot*, *Manihotoides* (Euphorbiaceae). New York, Organization Flora Neotropica, Monograph nº 13, Hafner Press. p. 1-272.

Santos J, Barreto MA, Oliveira FA, Vigna B, Souza AP. 2015. Microsatellite markers for *Urochloa humidicola* (Poaceae) and their transferability to other *Urochloa* species. BMC Research Notes 8: 83. doi: 10.1186/s13104-015-1044-9

Silva MJ, Alonso AA, Sodré RC. 2016. *Manihot pachycaulis* sp. nov. (Euphorbiaceae) from the Brazilian Cerrado. Nordic Journal of Botany 34: 60-65.

Silva MJ, Amaral US. 2020. Novelties in wild cassava (Manihot, Euphorbiaceae) from Brazil. Brittonia 72: 164-176.

Silva MJ, Soares TN, Oliveira PRO. 2017. Morphological characteristics and genetic evidence reveals a new species of *Manihot* (Euphorbiaceae, Crotonoideae) from Goiás, Brazil. PhytoKeys 77: 99-111.

Silva MJ, Sodré RC. 2014. A dwarf species of *Manihot* Mill.(Euphorbiaceae ss) from the highlands of Goiás, Brazil. Systematic Botany 39: 222-226.

Silva MJ. 2014. *Manihot veadeirensis* (Euphorbiaceae s. s.): a New species from the Brazilian Cerrado. Systematic Botany 39: 1161-1165.

Silva MJ. 2015. Two new wild cassava species (*Manihot*, Euphorbiaceae) from the Brazilian Cerrado. Phytotaxa 213: 131-139.

Silva MJ. 2016. *Manihot gratiosa* and *M. lourdesii* spp. nov. (Manihoteae, Euphorbiaceae) from the Brazilian Cerrado. Nordic Journal of Botany 34: 66-74.

Siqueira MVBM, Queiroz-silva JR, Bressan EA, *et al*. 2009. Genetic characterization of cassava (*Manihot esculenta*) landraces in Brazil assessed with simple sequence repeats. Genetics and Molecular Biology 32: 104-110.

Soares TN, Sant'Ana LL, Oliveira LK, Telles MPC, Collevatti RG. 2013. Transferability and characterization of microssatellite loci in *Anacardium humile* A. St. Hil. (Anacardiaceae). Genetics and Molecular Research 12: 3146-3149.

Taubert PHW. 1896. Polygalaceae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 21: 441-442.

Wagner HW, Sefc KM. 1999. IDENTITY 1.0 Centre for Applied Genetics. University of Agricultural Sciences, Vienna. http:/boku. ac. at/zag/forsgh/identity. htm. 10 Oct. 2020.

Weir BS, Evett I. W. 1998. Interpreting DNA evidence: statistical genetics for forensic scientists. Sunderland, MA, Sinauer Associates.

Weir BS. 1996. Genetic data analysis II: methods for discrete population genetic data. Sunderland, MA, Sinauer Associates.