Short Communication

Validation of EST-derived microsatellite markers for two Cerrado-endemic *Campomanesia* (Myrtaceae) species

E.A.G.C. Miranda[1], C.R.D. Boaventura-Novaes[2], R.S. Braga[1], E.F. Reis[3], J.F.N. Pinto[3] and M.P.C. Telles[1]

[1]Laboratório de Genética & Biodiversidade, Departamento de Genética, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brasil
[2]Programa de Pós-Graduação em Genética e Melhoramento de Plantas, Escola de Agronomia, Universidade Federal de Goiás, Goiânia, GO, Brasil
[3]Programa de Pós-Graduação em Agronomia, Universidade Federal de Goiás, Jataí, GO, Brasil

Corresponding author: M.P.C. Telles
E-mail: tellesmpc@gmail.com

Genet. Mol. Res. 15 (1): gmr.15017658
Received September 17, 2015
Accepted December 21, 2015
Published March 4, 2016
DOI http://dx.doi.org/10.4238/gmr.15017658

ABSTRACT. We assessed the transferability of 120 EST-derived *Eucalyptus* microsatellite primers to *Campomanesia adamantium* and *C. pubescens*. Both species are berry trees native to the Brazilian Cerrado, and population genetic information is poor. Twelve markers were used to analyze the genetic variability of four sampled populations. Regarding DNA extraction, we sampled leaf tissues from two populations of each species (80 individuals). Of the 120 primers evaluated, 87 did not amplify any PCR products, and 21 rendered nonspecific amplification. Twelve primers were successfully transferred, providing a low combined probability of genetic identity for both species (5.718×10^{-10} for *C. adamantium*; 1.182×10^{-11} for *C. pubescens*) and a high probability of paternity exclusion (0.99939 for *C. adamantium*; 0.99982 for *C. pubescens*). The average number of alleles in

the polymorphic loci was 6.8 for *C. adamantium* and 7.8 for *C. pubescens,* ranging from 2 to 16 alleles per locus. The observed heterozygosity values for *C. adamantium* and *C. pubescens* were 0.504 and 0.503, respectively, and the expected heterozygosity values for *C. adamantium* and *C. pubescens* were 0.517 and 0.579, respectively. The populations exhibited structured genetic variability with θP values of 0.105 for *C. adamantium* and 0.249 for *C. pubescens*. Thus, we concluded that these 12 microsatellite markers, transferred from *Eucalyptus*, were efficient for population genetic studies of *C. adamantium* and *C. pubescens*.

Key words: Gabiroba; Genetic diversity; SSR; Transferability

INTRODUCTION

Campomanesia adamantium O. Berg and *C. pubescens* DC. are two species of berry trees native to the Cerrado biome, and the genus is a member of the Myrtaceae family. *Campomanesia* plants are well-known berry trees whose fruits are called “gabiroba”, and they are utilized by local populations as edible fresh fruits or for culinary purposes in jellies, jams, ice creams, alcoholic beverages, and folk medicines (Ferreira, 1972). The genus also has considerable economic potential regarding bioactive compounds for pharmaceutical use (Czaikoski et al., 2015).

C. adamantium has interesting traits associated with its domestication, including variation in optimum harvest and consumption times (Santos et al., 2015), seed germination, and seed storage (Dresch et al., 2013, 2014). Previous morphological and molecular analyses of genetic diversity based on random amplified polymorphic DNA were conducted using progenies of 140 trees (de Assis et al., 2013). However, little information about the genetic diversity of *Campomanesia* is available. Additional population genetic studies are needed to support conservation and breeding programs, particularly since genetic variability in *Campomanesia* and other species is quickly being lost as Cerrado degradation continues. The biome is a hotspot for biodiversity conservation (Myers et al., 2000), and it is a key target for genetic variability maintenance and the sustainable use of genetic resources.

Microsatellites are one of the most widely used molecular markers in plants (Kalia et al., 2011). However, primer development for species with little or no genomic information is expensive. The conservation of transcribed regions between species allows the transference of expressed sequence tags (ESTs) that are derived from simple sequence repeat markers with a high success rate (Kalia et al., 2011). Several microsatellites have been transferred between different genera of the Myrtaceae family (Zucchi et al., 2002; Rai et al., 2013; Ferreira-Ramos et al., 2014; Nogueira et al., 2015), and this lowered the cost required for genetic diversity estimates. Therefore, the goal of this study was to investigate the heterologous amplification of microsatellite loci developed for *Eucalyptus* and to test their ability to genotype *C. adamantium* and *C. pubescens*.

MATERIAL AND METHODS

We analyzed 80 equally distributed samples from the Goiás State populations of Mineiros

(*C. adamantium*), Três Ranchos (*C. adamantium*), Santa Rita do Araguaia (*C. pubescens*), and Caiapônia (*C. pubescens*). Prior to the amplification of all of the collected samples, cross-amplification was tested in three *C. adamantium* individuals using 120 EST-derived *Eucalyptus* primers (Grattapaglia et al., 2015) (Table 1). Genomic DNA was extracted from leaf tissue using the cetyltrimethylammonium bromide 2% protocol (Doyle and Doyle, 1987). Polymerase chain reaction (PCR) was performed in a 10-μL final volume that contained 7.5 ng template DNA, 0.22 μM primers (forward + reverse), 0.23 μM dNTPs, 3.25 mg bovine serum albumin (25 mg/mL), 1X reaction buffer (10 mM Tris-HCl, pH 8.3, and $MgCl_2$), and 1 U Taq DNA polymerase. The following PCR program was used: an initial step of 5 min at 94°C; 35 cycles of 30 s at 94°C, 1 min at 48° to 62°C (depending on the primer), and 1 min at 72°C; and a final extension at 72°C for 45 min.

Polymorphisms were detected by running the samples on 6% denaturing polyacrylamide gels stained with silver nitrate. Each transferred forward primer was labeled with fluorescent dyes (5' HEX, 5' NED, or 5' 6-FAM) (Table 1). The lengths of the amplified products from the 80 samples were determined using an ABI3500 automated sequencer. Allele binning and calling were performed using Data Collection and GeneMapper 5.0 (Applied Biosystems) softwares, and null alleles were detected using MICRO-CHECKER version 2.2 (Van Oosterhout et al., 2004) software.

Analyses of genetic variability, including observed (H_O) and expected (H_E) heterozygosity values, Hardy-Weinberg equilibrium (HWE) (Nei, 1973), inbreeding coefficients (Weir and Cockerham, 1984), and linkage disequilibrium were performed using the Bonferroni correction included in the FSTAT 2.9.3.2 software (Goudet, 2001). The probability of genetic identity (I) (Paetkau et al., 1995) and the paternity exclusion probability (Q) (Weir, 1996) for each locus were estimated using the Identity 1.0 software (Wagner and Sefc, 1999).

RESULTS

Of the 120 tested primers, 87 (72.5%) lacked amplification, 21 (17.5%) amplified non-specific fragments, and 12 (10%) amplified clearly polymorphic alleles. The allele size of these 12 markers ranged from 199 to 384 bp, and the number of alleles ranged from 2 to 16. In *C. adamantium*, 82 alleles were amplified, with an average of 6.8 alleles per sample. Ninety-five alleles, with an average of 7.8 per locus, were amplified for *C. pubescens*. Regarding genotyping, it is possible to run three multiplexed reactions with four sets of primers each (Table 1). *C. adamantium* and *C. pubescens* loci EMBRA 1364 and EMBRA 1374 and *C. pubescens* loci EMBRA 1335, EMBRA 2011, EMBRA 809, and EMBRA 1470 showed significant heterozygote deficiencies based on the null allele analysis.

Average H_E and H_O values were 0.517 and 0.504 for *C. adamantium* and 0.579 and 0.503 for *C. pubescens*, respectively. *C. adamantium* and *C. pubescens* respectively exhibited five and seven loci that were not in Hardy-Weinberg equilibrium ($P < 0.05$). The combined probability of genetic identity values were 5.718×10^{-10} for *C. adamantium* and 1.182×10^{-11} for *C. pubescens*. The probability of paternity exclusion values were greater than 0.999 for both species. *C. adamantium* and *C. pubescens* exhibited one and nine loci pairs that significantly deviated from linkage equilibrium ($P > 0.05$), respectively. Furthermore, populations of both species have significantly structured genetic variability ($F = 0.306$, $\theta P = 0.105$ for *C. adamantium*; $F = 0.422$, $\theta P = 0.249$ for *C. pubescens*) (Table 2).

Table 1. Transferred microsatellites and amplification details for *Campomanesia adamantium* and *C. pubences*.

Locus	Repeat motif	Primer sequence	Ta	FD	M	AR	*Campomanesia adamantium*					*Campomanesia pubencens*				
							N_A	H_E	H_O	Q	I	N_A	H_E	H_O	Q	I
EMBRA 809	$(CT)_7$	F-5'-CCTCACGCCAAAAGAAGAAG-3' R-5'-GGGAATCGAAGAAACGATGA-3'	54	NED	1	199/227	8	0.731	0.625	0.487	0.122	6	0.614	0.324	0.430	0.159
EMBRA 1335	$(GTT)_5$	F-5'-TTGCTCCCATGATTACTCCC-3' R-5'-GTCTTCATCCTGGCAAGAGC-3'	56	HEX	1	314/338	2	0.025	0.025	0.012	0.951	7	0.730	0.500	0.475	0.126
EMBRA 1364	$(CTCC)_{15}$	F-5'-CGTTTTCGCTCCTCTCTCTC-3' R-5'-TGTAGAGATCGGGGTCCTTG-3'	59	FAM	1	305/345	16	0.927	0.750	0.829	0.013	12	0.741	0.625	0.552	0.091
EMBRA 2011	$(CTC)_4$	F-5'-AAAATACGACCGCCATGAAG-3' R-5'-TTGTGAGAGACGGAGACGTG-3'	58	NED	1	266/300	5	0.167	0.175	0.088	0.700	16	0.895	0.666	0.768	0.023
EMBRA 1363	$(GCC)_{15}$	F-5'-CCATAGCCCTCTGCTGATTC-3' R-5'-AATGGAAAATGGGTTCCTCC-3'	58	HEX	2	304/322	6	0.826	0.825	0.632	0.059	8	0.818	0.775	0.622	0.063
EMBRA 1374	$(CGCCGT)_{26}$	F-5'-GTCTGAACTCGGCTTCCTTG-3' R-5'-TTCTTCCCGTTGTAAATCCG-3'	55	FAM	2	354/384	8	0.788	0.256	0.579	0.078	7	0.692	0.325	0.434	0.152
EMBRA 1811	$(CTCCTG)_{26}$	F-5'-GTCGAGTTGAGTTCGCTTCC-3' R-5'-AGTGAATCGGGAGAGGAGGT-3'	62	NED	2	199/203	2	0.025	0.025	0.012	0.951	2	0.073	0.075	0.034	0.863
EMBRA 1868	$(TC)_{36}$	F-5'-TGTGGAGCATGGAGTAGCAG-3' R-5'-CAAATCTCAGAGACGCCACA-3'	62	NED	2	269/307	11	0.820	0.825	0.646	0.055	15	0.921	0.850	0.816	0.015
EMBRA 1076	$(AGG)_4$	F-5'-GCTGAACCTGATGGACCAGT-3' R-5'-CTTAGGGACCACCACCTTGA-3'	56	HEX	3	313/331	3	0.541	1.000	0.232	0.324	2	0.202	0.225	0.089	0.660
EMBRA 1362	$(TGC)_6$	F-5'-ACTTGATGGGTTCTCATCGC-3' R-5'-GGAAATCCTTACCACCAGCA-3'	59	HEX	3	270/296	10	0.832	0.832	0.653	0.053	7	0.814	0.700	0.617	0.065
EMBRA 1470	$(AG)_{10}$	F-5'-GCCAACCCCTCTAAAAGACC-3' R-5'-CAACTGCTACGACGTCCAAA-3'	56	FAM	3	321/329	3	0.142	0.125	0.068	0.746	5	0.584	0.175	0.358	0.215
EMBRA 1939	$(GCG)_6$	F-5'-AGATCTCATCCATGGCGTTC-3' R-5'-ACATCGCGATCTTCTTCGAC-3'	58	NED	3	228/252	8	0.643	0.700	0.426	0.164	7	0.582	0.800	0.343	0.227
Mean							6.83	0.539	0.504	0.999	5.718×10^{-10}	7.83	0.639	0.503	0.999	1.182×10^{-11}

Ta = annealing temperature (°C), FD = fluorescent dye, M = multiplex cluster, AR = allelic range, N_A = alleles number, H_E = expected heterozygosity, H_O = observed heterozygosity, Q = probability of paternity exclusion, I = probability of genetic identity.

Table 2. Genetic variability estimates for four *Campomanesia* populations.

	Populations					
	Mineiros	Três Ranchos	Mean	Santa Rita do Araguaia	Caiapônia	Mean
N_A	5.750	5.250	5.500	6.500	5.160	5.830
H_E	0.531	0.504	0.517	0.629	0.529	0.579
H_O	0.504	0.505	0.504	0.498	0.507	0.503
f	0.052	-0.002	0.025	0.213	0.042	0.138
	Campomanesia adamantium			*Campomanesia pubescens*		
F	0.306			0.422		
θP	0.105			0.249		

N_A = mean allele number, H_E = expected heterozygosity, H_O = observed heterozygosity, *f* = fixation index within population, *F* = population total fixation index, θP = genetic divergence between populations.

DISCUSSION

In general, the expected cross-amplification success rate between genera is about 10% (Barbará et al., 2007). Therefore, our primer transfer from *Eucalyptus* to *C. adamantium* and *C. pubescens* was effective and within expectations. However, high microsatellite transferability rates (up to 40.51%) from *Psidium guajava* to *Campomanesia* (*C. guaviroba*, *C. hirsuta*, and *C. phaea*) were reported (Nogueira et al., 2015). The differences in the results suggest that transferability varies between species and groups, and this is likely due to genetic proximity. The multiplexing of the 12 transferable primers into three quadruplexed reactions also saved time and resources.

The studied populations displayed significant population genetic structure, and no statistically significant inbreeding was detected. Within populations, variability accounted for the majority (~75%) of the total variation, and both species exhibited high values of H_O and mean genetic diversity. However, higher genetic diversity was observed among *C. pubescens* individuals compared to *C. adamantium* individuals, and this is relevant information for the establishment of effective population conservation, management, and breeding strategies. Furthermore, the successfully transferred microsatellite loci will be useful in future population genetic studies of these and other *Campomanesia* species.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Our research program in population genetics of Cerrado plants was supported by the “Núcleo de Excelência em Genética e Conservação de Espécies do Cerrado” - GECER project (PRONEX/FAPEG/CNPq #CP07-2009; #CH031/2010) and by several grants and fellowships from the GENPAC (“Geographical Genetics and Regional Planning for Natural Resources in Brazilian Cerrado”) research network, CNPq/MCT/CAPES/FAPEG, and the CNPq project (Proc. #563839/2010-4). E.F. Reis and M.P.C. Telles were also supported by productivity grants from CNPq. We thank Dr. Dario Grattapaglia for kindly supplying all of the primers analyzed, Dr. Lázaro José Chaves for helping collect samples on expeditions, and Dr. Heleno Dias Ferreira for plant identification.

REFERENCES

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. http://dx.doi.org/10.1111/j.1365-294X.2007.03439.x

Czaikoski K, Mesomo MC, Krüger RL, Queiroga CL, et al. (2015). Extraction of *Campomanesia xanthocarpa* fruit using supercritical CO2 and bioactivity assessments. *J. Supercrit. Fluids* 98: 79-85. http://dx.doi.org/10.1016/j.supflu.2015.01.006

de Assis ES, Dos Reis EF, Pinto JFN, Contim LAS, et al. (2013). Genetic diversity of gabiroba based on random amplified polymorphic DNA markers and morphological characteristics. *Genet. Mol. Res.* 12: 3500-3509.http://dx.doi.org/10.4238/2013.March.11.7

Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.

Dresch DM, Scalon SDPQ, Masetto TE and Vieira MC (2013). Germinação e vigor de sementes de gabiroba em função do tamanho do fruto e semente. *Pesqui. Agropec. Trop*. 43: 262-271. http://dx.doi.org/10.1590/S1983-40632013000300006

Dresch DM, Scalon SDPQ, Masetto TE and Mussury RM (2014). Storage of *Campomanesia adamantium* (Cambess.) O. Berg seeds: influence of water content and environmental temperature. *Am. J. Plant Sci* 5: 2555-2565. http://dx.doi.org/10.4236/ajps.2014.517269

Ferreira MB (1972). Frutos comestíveis nativos do D.F.: gabirobas, pitangas e araçás. 1st edn. *Brasília* 4: 11-16.

Ferreira-Ramos R, Accoroni KAG, Rossi A, Guidugli MC, et al. (2014). Genetic diversity assessment for *Eugenia uniflora* L., *E. pyriformis* Cambess., *E. brasiliensis* Lam. and *E. francavilleana* O. Berg neotropical tree species (Myrtaceae) with heterologous SSR markers. *Genet. Resour. Crop Evol.* 61: 267-272. http://dx.doi.org/10.1007/s10722-013-0028-7

Goudet J (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at http://www.unil.ch/izea/softwares/fstat.html.

Grattapaglia D, Mamani EM, Silva-Junior OB and Faria DA (2015). A novel genome-wide microsatellite resource for species of *Eucalyptus* with linkage-to-physical correspondence on the reference genome sequence. *Mol. Ecol. Resour.* 15: 437-448. http://dx.doi.org/10.1111/1755-0998.12317

Kalia RJ, Rai MK, Kalia S, Singh R, et al. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica* 177: 309-334. http://dx.doi.org/10.1007/s10681-010-0286-9

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, et al. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858. http://dx.doi.org/10.1038/35002501

Nei M (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* 12: 3321-3323.

Nogueira AM, Ferreira A and Ferreira MFS (2015). Transferability of microsatellites from *Psidium guajava* to *Eugenia, Myrciaria, Campomanesia*, and *Syzygium* species (Myrtaceae). *Plant Mol. Biol. Rep.* 34: 249-256.

Paetkau D, Calvert W, Stirling I and Strobeck C (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* 4: 347-354. http://dx.doi.org/10.1111/j.1365-294X.1995.tb00227.x

Rai MK, Phulwaria M and Shekhawat NS (2013). Transferability of simple sequence repeat (SSR) markers developed in guava (*Psidium guajava* L.) to four Myrtaceae species. *Mol. Biol. Rep.* 40: 5067-5071. http://dx.doi.org/10.1007/s11033-013-2608-1

Santos MA, Megguer CA, Costa AC and Lima JS (2015). Growth and development of gabiroba *Campomanesia adamantium* (Cambess.) O. Berg fruits. *Afr. J. Agric. Res.* 10: 1765-1772. http://dx.doi.org/10.5897/AJAR2014.8517

Van Oosterhout C, Hutchinson WF and Wills DPM (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535-538. http://dx.doi.org/10.1111/j.1471-8286.2004.00684.x

Wagner HW and Sefc KM (1999). Identity 1.0-Freeware program for the analysis of microsatellite data. Centre for Applied Genetics, University of Agricultural Sciences, Vienna.

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

Weir BS and Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370. http://dx.doi.org/10.2307/2408641

Zucchi MI, Brondani RPV, Pinheiro JB, Brondani C, et al. (2002). Transferability of microsatellite markers from *Eucalyptus* spp. to *Eugenia dysenterica* (Myrtaceae family). *Mol. Ecol. Notes* 2: 512-513. http://dx.doi.org/10.1046/j.1471-8286.2002.00297.x