GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Transferability and characterization of microssatellite loci in *Anacardium humile* A. St. Hil. (Anacardiaceae)

T.N. Soares, L.L. Sant'Ana, L.K. de Oliveira, M.P.C. Telles and R.G. Collevatti

Laboratório de Genética e Biodiversidade, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brasil

Corresponding author: T.N. Soares
E-mail: tnsoares@gmail.com

Genet. Mol. Res. 12 (3): 3146-3149 (2013)
Received June 1, 2012
Accepted September 25, 2012
Published January 4, 2013
DOI http://dx.doi.org/10.4238/2013.January.4.24

ABSTRACT. Microsatellite markers were transferred from the cashew, *Anarcadium occidentale*, to *Anacardium humile* (Anacardiaceae), a Neotropical shrub from the Brazilian savanna, that produces an edible nut and pseudo-fruit. We tested 14 microsatellite primers from *A. occidentale* on *A. humile*. Polymorphism of each microsatellite locus was analyzed based on 58 individuals from three populations. Twelve loci amplified successfully and presented 2 to 9 alleles; expected heterozygosity ranged from 0.056 to 0.869. These 12 microsatellite loci provide a new tool for the generation of fundamental population genetic data for devising conservation strategies for *A. humile*.

Key words: *Anacardium humile*; *Anarcadium occidentale*; Genetic diversity; Heterologous primer; Neotropical savannas

INTRODUCTION

Anacardium humile A. St. Hil. (Anacardiaceae) is a Neotropical shrub species distributed in well-delimited patches of rocky savannas in the Cerrado biome, Central-West Brazil. The nut, similar to the Brazilian cashew nut, and the edible pseudo-fruit are consumed *in natura* or used as a source of raw material by small industries of traditional candies, and also for homemade therapeutic recipes due to its antifungal, antibacterial and antidiarrheal activity, thereby playing an important role in the traditional culture and economy of the local population of Central-West Brazil. However, no molecular markers to date are available for population genetic studies and to clarify the evolutionary mechanisms involved in the distribution of genetic variability in this important genetic resource.

Transferability of microsatellite loci between closely related species reduces the cost of primer development, opening new perspectives for the development of population genetic studies. A high rate of transferability has already been reported for different plant species (e.g., Collevatti et al., 1999; Barbará et al., 2007; Braga et al., 2007; Kriedt et al., 2011).

We are interested in understanding the population genetic structure and gene flow in *A. humile* and obtaining useful information for the development of conservation strategies. Herein, we present the results of the transferability of 12 polymorphic microsatellite loci from *A. occidentale* L. to *A. humile*.

MATERIAL AND METHODS

For transferability analysis, leaves from three individuals of *A. humile* were sampled, and DNA was extracted following the CTAB protocol (Doyle and Doyle, 1990). From 21 primers previously developed for *A. occidentale* (Croxford et al., 2006), we selected the 14 most variable (Table 1) to test in *A. humile*. PCR (polymerase chain reaction) was performed in a 15-µL reaction volume containing 12 ng template DNA, 3.8 µM of each primer, 1 U Taq DNA polymerase (Phoneutria, Belo Horizonte, MG, Brazil), 250 µM of each dNTP, 0.25 µg BSA and 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$). For amplifications, we varied the annealing temperature from 56° to 66°C, under the following conditions: 94°C for 5 min (one cycle); 94°C for 1 min, 56° to 66°C (annealing temperature) for 1 min, and 72°C for 1 min (30 cycles); and 72°C for 7 min (one cycle). Amplifications were checked on 1% agarose gels.

For those loci that amplified successfully, we analyzed the polymorphism in 58 individuals from three populations: BAMMG, Bambuí, MG (S20°06'02.4", W45°57'25.1"); MIMGO, Mimoso, GO (S15°02'55.9", W48°08'49.0"); BARBA, Barreiras, BA (S12°07'05.5", W45°11'52.5"). DNA extraction and amplification followed the same protocols described above, but with the annealing temperature optimized in the test procedures (Table 1). Polymorphisms were detected on 6% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001) and sized by comparison to a 10-bp DNA ladder standard (Invitrogen, Grand Island, NY, USA). Statistical analyses were performed with the FSTAT 2.9.3.2 software (Goudet, 2002).

RESULTS AND DISCUSSION

Twelve microsatellite loci clearly amplified interpretable fragments using a single PCR

protocol (Table 1) and were polymorphic in the three populations analyzed (Table 2). *A. humile* showed higher levels of polymorphism than did *A. occidentale* (Croxford et al., 2006), with 2 to 9 alleles per locus and expected heterozygosity ranging from 0.056 to 0.869 (Table 2).

Table 1. Microsatellite primers tested for *Anacardium humile* A. St. Hil.

Locus	Sequence (5'-3')	Repeat	Size range (bp)	Ta (°C)
mAoR2	GGCCATGGGAAACAACAA GGAAGGGCATTATGGGTAAG	$(CA)_{10}(TA)_6$	350-380	60
mAoR3	CAGAACCGTCACTCCACTCC ATCCAGACGAAGAAGCGATG	$(AC)_{12}(AAAAT)_2$	236-256	60
mAoR6	CAAAACTAGCCGGAATCTAGC CCCCATCAAACCCTTATGAC	$(AT)_5(GT)_{12}$	140-156	60
mAoR11	ATCCAACAGCCACAATCCTC CTTACAGCCCCAAACTCTCG	$(AT)_3(AC)_{16}$	226-242	62
mAoR12	TCACCAAGATTGTGCTCCTG AAACTACGTCCGGTCACACA	$(AC)_{12}ARAC(AT)_4$	318-340	60
mAoR16	GGAGAAAGCAGTGGAGTTGC CAAGTGAGTCCTCTCACTCTCA	$(GT)_8(TA)_{17}(GT)_3$	222-260	60.3
mAoR17	GCAATGTGCAGACATGGTTC GGTTTCGCATGGAAGAAGAG	$(GA)_{24}$	138-156	58
mAoR29	GGAGAAGAAAAGTTAGGTTTGAC CGTCTTCTTCCACATGCTTC	$(TG)_{10}$	304-322	58.2
mAoR33	CATCCTTTTGCCAATTAAAAACA CACGTGTATTGTGCTCACTCG	$(CT)_{18}(AT)_{19}$	-	*
mAoR35	CTTTCGTTCCAATGCTCCTC CATGTGACAGTTCGGCTGTT	$(AG)_{14}$	148-158	60
mAoR41	GCTTAGCCGGCACGATATTA AGCTCACCTCGTTTCGTTTC	$(GGT)_8$	151-157	60
mAoR42	ACTGTCACGTCAATGGCATC GCGAAGGTCAAAGAGCAGTC	$(CAT)_9TAT(CTT)_7$	187-208	62
mAoR52	GCTATGACCCTTGGGAACTC GTGACACAACCAAAACCACA	$(GT)_{16}(TA)_2$	186-204	60
mAoR59	TCCGCCCCTACTCCTATATT TGGTGTCGACTGCTTCTTGT	$(AT)_7(GT)_{14}$	-	*

Data are reported for 58 individuals from three populations. Ta = annealing temperature. *Amplification failed for the range of annealing temperature used in the present study.

Table 2. Genetic characterization of 12 microsatellite loci in three populations of *Anacardium humile* A. St. Hil.

Locus	BAMMG				MIMGO				BARBA			
	N	N_A	H_E	H_O	N	N_A	H_E	H_O	N	N_A	H_E	H_O
mAoR2	16	5	0.583	0.375	18	6	0.775	0.890	24	6	0.745	0.624
mAoR3	16	9	0.869	0.438	18	6	0.747	0.500	24	8	0.678	0.542
mAoR6	16	6	0.831	0.312	18	5	0.794	0.111	24	5	0.791	0.417
mAoR11	16	9	0.842	0.813	18	6	0.732	0.722	20	4	0.553	0.000
mAoR12	16	4	0.688	0.438	18	7	0.824	0.445	24	8	0.836	0.416
mAoR16	16	3	0.567	0.250	18	5	0.776	0.222	18	4	0.348	0.278
mAoR17	16	7	0.752	0.812	17	4	0.563	0.412	24	5	0.717	0.333
mAoR29	16	6	0.831	0.500	18	4	0.588	0.222	23	7	0.539	0.392
mAoR35	16	2	0.483	0.499	18	3	0.109	0.111	24	4	0.122	0.125
mAoR41	16	2	0.467	0.438	18	2	0.056	0.056	24	2	0.120	0.125
mAoR42	16	7	0.567	0.500	18	5	0.727	0.611	24	5	0.724	0.833
mAoR52	16	4	0.606	0.125	18	8	0.835	0.118	24	7	0.729	0.208

N = number of individuals genotyped; N_A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity. BAMMG = Bambuí-MG; MIMGO = Mimoso-GO; BARBA = Barreiras-BA.

This was most likely due to the origin of the individuals used to characterize the microsatellites in *A. occidentale*, from a seed orchard, and the high relationship between them (Croxford et al., 2006). The twelve transferred loci also showed a high combined probability of paternity exclusion (QC = 0.999968861954), which corresponds to the power with which a locus excludes an individual of being the parent of an offspring (Weir, 1996), and very low combined probability of identity (IC = 1.765115 x 10^{-13}), which corresponds to the probability of two random individuals displaying the same genotype (Chakravarat and Li, 1983).

The 12 microsatellite loci transferred for *A. humile* in this study are highly polymorphic and together display suitable values of probability of genetic identity and paternity exclusion to readily discriminate individuals and also to detail parentage analysis, opening a new perspective for population genetic analyses in *A. humile*.

ACKNOWLEDGMENTS

Research supported by FAPEG/AUX PESQ CH 007/2009; CNPq/FAPEG-GO (GENPAC 2 #563839/2010-4) and CNPq (#472717/2011-1). T.N. Soares, M.P.C. Telles and R.G. Covellatti's overall research program in molecular ecology have been continuously supported by CNPq and CAPES grants and fellowships.

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.

Braga AC, Reis AM, Leoi LT, Pereira RW, et al. (2007). Development and characterization of microsatellite markers for the tropical tree species *Tabebuia aurea* (Bignoniaceae). *Mol. Ecol. Notes* 7: 53-56.

Chakravarat I and Li CC (1983). The Effect of Linkage on Paternity Calculations. In: Inclusion Probabilities in Parentage Testing (Walker RH, ed.). American Association of Blood Banks, Arlington, 411-420.

Collevatti RG, Brondani RV and Grattapaglia D (1999). Development and characterization of microsatellite markers for genetic analysis of a Brazilian endangered tree species *Caryocar brasiliense*. *Heredity* 83: 748-756.

Creste S, Tulmann Neto A and Figueira A (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Mol. Biol. Rep.* 19: 299-306.

Croxford AE, Robson M and Wilkinson MJ (2006). Characterization and PCR multiplexing of polymorphic microsatellite loci in cashew (*Anacardium occidentale* L.) and their cross-species utilization. *Mol. Ecol. Notes* 6: 249-251.

Doyle JJ and Doyle JL (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Goudet J (2002). FSTAT 2.9.3.2.: A Program to Estimate and Test Gene Diversities and Fixation Indices. Available at [http://www.unil.ch/izea/softwares/fstat.html]. Accessed April 30, 2012.

Kriedt RA, Ramos-Fregonezi AM, Beheregaray LB, Bonatto SL, et al. (2011). Isolation, characterization, and cross-amplification of microsatellite markers for the *Petunia integrifolia* (Solanaceae) complex. *Am. J. Bot.* 98: e277-e279.

Weir BS (1996). Genetic Data Analysis II. Sinauer Associates, Sunderland.