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Microsatellites for mahoganies: Twelve new loci for *Swietenia macrophylla* and its high transferability to *Khaya senegalensis*[1]

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- *Premise of the study:* A new set of 12 microsatellite markers was developed and characterized for big-leaf mahogany (*Swietenia macrophylla* King, Meliaceae) and its transferability assayed in the African mahogany, *Khaya senegalensis* (Desr.) A. Juss. (Meliaceae), to study population and conservation genetics of these threatened tropical timber species.
- *Methods and Results:* Using an enriched library approach twelve novel microsatellite loci were identified for *S. macrophylla*. The number of alleles per locus ranged from 5 to 14 and mean observed and expected heterozygosities were 0.819 and 0.822, respectively. Twenty microsatellite loci developed for *S. macrophylla* (12 from this study and eight previously published) were tested for *K. senegalensis* and 10 polymorphic were characterized.
- *Conclusions:* The results show the highly informative content of the new SSR loci for *Swietenia macrophylla* and the high effectiveness of these microsatellites for population genetics, gene flow, and mating system studies in *Khaya senegalensis.*

Key words: cross-amplification; *Khaya senegalensis*; Meliaceae; SSR loci; *Swietenia macrophylla*; threatened tropical trees.

The mahoganies are highly valuable members of the Meliaceae family, and naturally distributed in the Neotropics and Africa (Pennington 1981). The big-leaf mahogany (*Swietenia macrophylla*) is the most valuable and over-exploited Neotropical timber species. The African mahogany, *Khaya senegalensis*, is heavily harvested by indigenous people in West Africa also for its timber, bark and foliage (Gaoue and Ticktin, 2007). Due to over exploitation the conservation status of both species has been a subject of increasing concern. The extent of over-harvesting on mahoganies suggests that this may have affected the genetic diversity and structure of their populations.

Microsatellite, or simple sequence repeats (SSR), have been widely recognized as powerful and informative genetic markers. The variability observed at SSR loci allows the precise genetic identification of individuals in natural populations, as well as the estimation of genetic parameters that have fundamental importance in understanding the genetic impacts of timber extraction, harvest of nontimber forest products, and for the genetic conservation and management of heavily exploited tropical trees such as *S. macrophylla* and *K. senegalensis.* We develop and characterize a set of twelve new microsatellite markers for big-leaf mahogany (*S. macrophylla*) and successfully transfer these loci to African mahogany (*K. senegalensis*).

METHODS AND RESULTS

Twenty-four clones were isolated from an enriched genomic library previously constructed for *Swietenia macrophylla* (Lemes et al., 2002). These clones were evaluated to obtain additional informative SSR loci for *S. macrophylla* primarily for timber tracking application. The analysis of the 24 clone sequences showed that four of them were redundant. They were eliminated from the genetic characterization. Of the 20 remaining *S. macrophylla* SSR loci, 12 were selected for genetic analysis based on the quality and robustness of the amplified products resolved in 3% agarose gel.

Leaf material for DNA extraction was collected from 20 individuals from four populations (5 ind/pop) of *S. macrophylla* previously collected in the Brazilian Amazon (Lemes et al., 2003) and from 237 individuals from 12 populations of *K. senegalensis* in Benin, West Africa (Gaoue and Ticktin, 2007) for the transferability tests. Leaves were preserved in silica gel and maintained at –20°C until DNA extraction. Genomic DNA was extracted using a standard CTAB protocol (Doyle and Doyle, 1987). PCR amplification was carried out in a final reaction volume of 13 µl containing 0.9 µM of each primer, 1 unit Taq DNA polymerase, 200 µM of each dNTP, 1× reaction buffer (10 mM

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Tris-HCl, pH 8.3, 50 mM NH_4, 1.5 mM $MgCl_2$), BSA (Bovine Serum Albumine – 2.5 mg/ml), 7.5 ng of template DNA, ultrapure water, for posterior analysis in silver stained PAGE (Polyacrylamide Gel Electrophoresis), and 1.25 μM each forward and reverse primers, for fluorescent based analysis in an ABI 377XL sequencer. PCR amplifications were performed using the following program: 94°C for 5 min followed by 30 cycles of 94°C for 1 min, annealing temperature for each locus (see Tables 1 and 2) for 1 min and 72°C for 1 min; and a final elongation step at 72°C for 7 min. The PCR products were visualized in 2% agarose gels containing 0.1 μg/ml of ethidium bromide in 1× TBE buffer (89 mM Tris-borate, 2mM EDTA pH 8.3) and sized with a 1Kb DNA ladder (Gibco, MD). The primers that showed clear and robust band amplification in agarose gel were selected for analysis of polymorphisms. For the first set of twelve SSR loci presented here for *S. macrophylla*, polymorphism analysis (for both species of mahogany) was conducted in 4% PAGE stained with silver nitrate (Creste et al., 2001) and sized by comparison to a 10 bp DNA ladder (Gibco, MD). For the second set of eight SSR loci (sm01, sm22, sm31, sm32, sm40, sm46, sm47, sm51), previously published in Lemes et al. (2002), used here only in the transferability analysis for *K. senegalensis*, amplified products were resolved in 5% PAGE in an ABI 377XL sequencer as described in Lemes et al. (2003). Allele sizes were estimated using GeneScan and Genotyper (ABI, Inc). We estimated number of alleles, and expected and observed heterozygosities for each locus and averaged over all loci using FSTAT program (Goudet, 2000). Two parameters of genetic information content were also estimated for each locus and combined for all loci: (1) probability of genetic identity (I), which represents the probability that two random individuals from a population would have identical genotypes and (2) paternity exclusion probability (Q), which corresponds to the ability of a locus to exclude a random individual from paternity, as described in Lemes et al. (2002).

We identified a total of 124 alleles over 12 SSR loci in 20 adult individuals of *S. macrophylla* sampled from four wild populations, with an average of 10.3 alleles (range 5-14) per locus. The mean expected and observed heterozygosities were 0.822 and 0.819, respectively. The combined probability of identity was 1.44×10^{-15} and combined probability of paternity exclusion was 0.9999977 over all loci (Table 1).

From the 20 SSR loci developed for *S. macrophylla* (12 from this study and eight from Lemes et al., 2003), all loci (100%) successfully amplified for *K. senegalensis*. However, five (sm31, sm32, sm39, sm48, and sm51) showed monomorphic after tests for polymorphisms in silver stained PAGE. Hence the percentage of informative loci tested for *K. senegalensis* was 75%. From these informative loci, we selected ten that showed very clean and easily interpretable bands, for further characterization as part of a study on the effect of bark and foliage harvest on the genetic diversity and population structure of *K. senegalensis*. From this set of ten SSR loci, we successfully amplified a total of 108 alleles with an average of 10.8 alleles per locus. The mean observed (H_o) and expected heterozygosities (H_e) over the 10 loci were 0.486 and 0.484, respectively (Table 2). The probability of exclusion ranged from 0.018 and 0.585 with a combined probability of paternity exclusion (QC) of 0.9999994. The combined probability of genetic identity IC was 1.22×10^{-7}.

In contrast to Sexton et al. (2010) our study showed very high levels of polymorphism and transferability of the microsatellite

Table 1. Characteristics of 12 new microsatellite markers developed for *Swietenia macrophylla*. SSR loci names; repeat motifs; primer sequences (F: forward, and R: reverse); T_a, annealing temperature in °C; allelic size range in base pairs (bp); A, number of alleles per locus; H_e, expected heterozigosity; H_o, observed heterozigosity; I, probability of genetic identity; Q, probability of paternity exclusion; IC, combined probability of genetic identity; QC, combined probability of paternity exclusion; and Genbank accession numbers.

SSR Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Allelic size range (bp)	A	H_e	H_o	I	Q	GenBank Accession Numbers
sm05	$(AG)_{17}GG(AG)_6$	F: GCATGAGCTTGAGAGAATC R: CAGAGGACTGAAGTAGCTGA	60	240–262	8	0.772	1.000	0.0953	0.4357	HM041033
sm07	$(AG)_{12}TT(AG)_7$	F: GATAGCGGAGCCGGTGATT R: GGATGGAAGGCTCAAGATTCG	60	240–250	5	0.787	0.579	0.1002	0.4473	HM041034
sm08	$(AG)_{17}$	F: TTCCTCTTCCTTGACCGCTC R: CGTACGGTTATGATCAGCGAC	55	238–262	9	0.806	0.555	0.0754	0.3925	HM041035
sm12	$(AG)_{24}$	F: AGAGTGTTCGAGAGCCTCAA R: AGAGCCGAATTCACCGAT	56	196–224	10	0.827	0.750	0.0653	0.3628	HM041038
sm18	$(AG)_{19}$	F: CTGTCATGCATATCGTTGGA R: GGGCAGATAAAGAGGAACAAG	56	196–232	14	0.889	1.000	0.0288	0.2455	HM041039
sm20	$(AG)_{28}$	F: CAACTCGTGAGGAATTTACC R: TGGGATTTGTGTTCACCTT	56	158–200	14	0.900	1.000	0.0248	0.2287	HM041046
sm28	$(AG)_{25}$	F: GCTCGGTGGTGTTACAGTT R: CAGTATGACAGTATCAAGGGGA	56	126–166	11	0.793	0.850	0.0682	0.3803	HM041040
sm36	$(AG)_{19}$	F: CGTGGTTGCTACCTATATGC R: TATACCGACCGCGTTAAGT	56	200–238	11	0.803	0.650	0.0705	0.4076	HM041041
sm39	$(AG)_{19}$	F: CAGTCATGGAGCGTAGCTAA R: TGCAGTTTCAGAACCTGAATC	56	154–202	13	0.861	1.000	0.0417	0.2934	HM041042
sm43	$(CT)_{18}$	F: TAGGAACCAACCACCAAC R: GTTCTCCTGCTCTCTTTGA	56	210–238	7	0.741	0.900	0.1160	0.4762	HM041043
sm48	$(AG)_{20}$	F: TCAGGAATGGAAGGTACAGG R: CAGTCATGGAGCGTAGCTAA	56	264–310	9	0.762	0.600	0.0943	0.4385	HM041044
sm49	$(AG)_{19}$	F: GAACTGGCAATGTGCTGACT R: TCGGCAATAGCAAGACATTC	64	136–174	13	0.920	0.947	0.0196	0.1535	HM041045
Mean			—	—	10.3	0.822	0.819	IC = 1.44×10^{-15}	QC = 0.9999977	

Table 2. Characteristics of 10 microsatellite loci developed for *Swietenia macrophylla* and successfully transferred to *Khaya senegalensis* based on analysis of 12 populations. SSR loci names; N, sample size; allelic size range in base pairs (bp); T_a, annealing temperature in °C; R_s, allelic richness; A, number of alleles per locus; H_e and H_o, expected and observed heterozygosities; I, probability of genetic identity; Q, probability of paternity exclusion; IC, combined probability of genetic identity; and QC combined probability of paternity exclusion. Values in brackets are standard deviations.

SSR Locus	N	Alleles Size (bp)	*Ta* (°C)	R_s	A	H_e	H_o	I	Q
sm01	234	258–280	56	3.229	6	0.521 (0.132)	0.420 (0.131)	0.258	0.319
sm46	237	190–200	56	3.056	5	0.406 (0.214)	0.375 (0.220)	0.341	0.25
sm05	230	222–240	60	5.387	10	0.577 (0.135)	0.514 (0.171)	0.179	0.414
sm07	206	240–250	52	2.948	6	0.575 (0.049)	0.979 (0.040)	0.291	0.295
sm28	237	140–160	52	1.297	5	0.034 (0.056)	0.009 (0.021)	0.932	0.018
sm22	232	116–146	62	2.787	10	0.287 (0.162)	0.217 (0.157)	0.506	0.166
sm12	237	118–182	56	2.247	4	0.383 (0.130)	0.257 (0.156)	0.426	0.192
sm18	231	158–248	52	4.993	17	0.649 (0.064)	0.598 (0.128)	0.163	0.451
sm08	205	226–284	56	7.088	24	0.741 (0.084)	0.747 (0.129)	0.078	0.585
sm36	232	218–262	52	5.677	21	0.669 (0.087)	0.752 (0.168)	0.0104	0.494
Mean				3.871	10.8	0.484 (0.212)	0.486 (0.292)	IC =1.22 x 10^{-7}	QC = 0.9999994

loci isolated from *S. macrophylla* and tested for *K. senegalensis*. It is probably related to the higher number of *S. macrophylla* SSR loci suitable to test for *K. senegalensis* here (20) compared to their study (8). Informative SSR transferability in plants is mostly restricted to congeners. One possible reason for the successful cross-genera informative amplification of the SSR markers is related to the close phylogenetic relatedness of the two genera *Khaya* and *Swietenia* (Muellner et al., 2003).

CONCLUSIONS

The new 12 SSR markers enhance the set of informative molecular markers available for application on population genetics, conservation, and timber tracking in *S. macrophylla*. The results also demonstrated very high level of transferability of SSR loci between mahogany genera suggesting not only cross-genera amplification but also conservation of loci among two species across two continents that were previously connected. The successful transferability of the *S. macrophylla* SSR markers to *K. senegalensis* represents a potential for immediate application on studies of genetic diversity and population structure, gene flow and mating system of African mahogany's populations. Such information will provide important insights to develop effective conservation and sustainable management for this valuable species.

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