Isolation and characterization ofmicrosatellitemarkers for Acacia senegal (L.)Willd., amultipurpose arid and semi-arid tree

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Isolation and characterization of microsatellite markers for *Acacia senegal* (L.) Willd., a multipurpose arid and semi-arid tree

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Abstract

***Acacia senegal* is a multipurpose African tree that improves the soil fertility of degraded areas. The species is exploited mainly for gum arabic, but it also supplies fuel wood and fodder for animals. Despite its wide distribution in Africa, no microsatellite markers have yet been characterized for this species. In this study, we characterized 11 polymorphic microsatellite loci specifically designed for *A. senegal* and analysed 247 individuals from three populations from Niger. On average, 10.9 alleles per locus were detected and expected heterozygosity ranged from 0.160 to 0.794, showing the ability of the markers to detect genetic diversity in this species.**

Keywords: *Acacia senegal*, conservation, genetic diversity, Leguminosae, microsatellite, molecular markers

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Acacia is the second largest genus in the family Leguminosae with about 1350 species (Maslin 2003). The current classification of *Acacia* differentiates three subgenera (Ross 1979): *Acacia*, *Heterophylum* and *Aculeiferum*. *Acacia senegal* belongs to the *Aculeiferum* subgenus. *Acacia senegal* is a multipurpose African tree that has been highly valued for centuries for its gum arabic. Today *A. senegal* is grown primarily for gum, but among the poorest rural populations of the Sahelian zone, it plays a major part in restoring soil fertility and providing fuel and fodder. Climate change threatens the genetic variability of *A. senegal*, which disappears when the annual rainfall is below 300 mm. In these changeable environmental conditions, it is essential to characterize the genetic diversity of this species from an adaptive point of view to implement a strategy for sustainable management.

Genetic diversity of *A. senegal* has been assessed using isoenzymatic markers in a population from Senegal (Chevallier *et al.* 1994) and random amplified polymorphic DNA and intersimple sequence repeat markers in Kenyan populations (Chiveu *et al.* 2008). However, to our knowledge, despite economic and ecological importance of *A. senegal*, no highly polymorphic and co-dominant markers, such as microsatellites (simple sequence repeats, SSRs), have been yet characterized for this species. In this study, we characterize 11 polymorphic SSR loci specifically designed for *A. senegal*.

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A genomic library enriched for (CT)n, (GT)n, (ATT)n, (CTT)n and (ATG)n was developed according to the protocol described by Billotte *et al.* (1999) with some modifications. The methodology was based on biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. Total genomic DNA was extracted from the leaves of one individual sampled in a provenance test near Niamey. Total purified DNA (7.7 μg) was digested with *Alu*I and the resulting solution of restriction fragments was enriched in (CT)n, (GT)n, (ATT)n, (CTT)n and (ATG)n repeats. Microsatellite-enriched DNA fragments were ligated into pGEM-T Easy vector (Promega) and used to transform XL1-Blue competent *Escherichia coli* cells (Stratagene). Positive colonies (blue/-white β-galactosidase selection) were tested by polymerase chain reaction (PCR) to check for the presence of inserts. A southern blot was performed to check the presence of microsatellites in the amplified fragments by hybridization with γ-32P-labelled oligoprobes. Bacterial clones containing inserts of sizes from 500 to 800 bp were selected and cultured. A total of 96 recombinant colonies were selected and sequenced. Forty-eight clones containing a SSR motif were identified. Primer pairs were designed for 43 putative loci using primer design software, OLIGO-EPLORER (http://www.genelink.com/tools/

Table 1 Characteristics of 11 microsatellite markers for *Acacia senegal*

Locus name	Accession no. EMBL GenBank	Motif repeat	Primer sequence (5′–3′)	T_a (°C)	Allele size range (bp)	N_a	N	R Ba	R Do	R Té	H_O Ba	H_O Do	H_O Té	H_E Ba	H_E Do	H_E Té
mAsCIRB09	FM883654	(TA)3 TG (TA)3 TG (TA)3	F: *CCTTATGAACCAAAACCAGC R: CAACGAGCATTAACACCAAC	53.8	297–341	7	239	1.98	5.00	3.48	0.016^{NS}	0.409^{NS}	0.044^{NS}	0.016	0.399	0.044
mAsCIRB10	FM883648	(TA)7 (N)6 (CA)5 (N)6 (CA)4 (N)4 (TA)3	F: *TTTCTCCCCATAAATCGTGC R: TTATCCGTTTGCTTGCCG	52.8	163–197	13	245	7.00	10.07	7.83	0.850^{NS}	0.787^{NS}	0.747^{NS}	0.805	0.823	0.754
mAsCIRC07	FM883653	(TG)9 TC (TA)3	F: *TAATCGTCCGTAGCAAG R: TCCTATTCTCCTCCCTC	49.1	148–172	11	214	7.98	6.82	7.61	0.750^{NS}	0.184*	0.741^{NS}	0.674	0.502	0.740
mAsCIRE06	FM883647	(GA)8 G (GA)7	F: *CTGTGATGGATAACCTTTTG R: TCCTCTTCCTCCTTCTCTC	52	168–190	9	237	4.98	4.00	6.17	0.516^{NS}	0.465^{NS}	0.681^{NS}	0.531	0.445	0.672
mAsCIRE07	FM883644	(GGA)2 (GAA) (GGA)4	F: *GAAGCAGAAGCAGAAGCAGC R: CCCCCCTCACACTCATCTC	55.9	148–163	6	244	2.00	5.22	2.65	0.050^{NS}	0.800^{NS}	0.066^{NS}	0.050	0.671	0.065
mAsCIRE08	FM883650	(TG)5 (AG)9	F: *CCTCACGAATTGGATATGTC R: GAGAACGATGGCTTTGTG	53.7	146–182	12	247	4.98	7.30	6.13	0.400^{NS}	0.677^{NS}	0.637^{NS}	0.409	0.677	0.574
mAsCIRE10	FM883645	(GAT)6	F: *GAGAAACTGGAGAGGGGAAG R: GCGACAAAAGTAGTAAAGGGC	52.5	117–132	5	241	2.00	4.95	2.00	0.440^{NS}	0.505*	0.404^{NS}	0.413	0.698	0.463
mAsCIRF02	FM883652	(GT)11	F: *TAGTGACAACCGCATCTC R: CAGTCAAATACACGCAAAC	51.6	210–292	25	246	5.00	21.43	6.27	0.550^{NS}	0.823^{NS}	0.611^{NS}	0.667	0.902	0.644
mAsCIRF03	FM883651	(TA)4 (N)55 (AC)10	F: *CACTTTTACTTTGTGATCTCCC R: CAGTCTTGTGTGCGTCTTAC	52.5	272–336	9	247	3.98	6.55	4.17	0.483^{NS}	0.458^{NS}	0.296^{NS}	0.489	0.498	0.305
mAsCIRH01	FM883646	(TG)9 TA CA (TA)3	F: *CAGAGGTTCAGGTATATGTGTG R: TTGTGTTAGTTTCAGATGGC	52.2	192–236	15	246	12.97	10.78	8.17	0.816^{NS}	0.736^{NS}	0.626^{NS}	0.852	0.749	0.635
mAsCIRH09	FM883649	(GAA)6	F: *CCACTCCAGGTACAAATCAC R: AGCCAGCAGAAAACTTAGG	49.9	135–156	8	246	8.00	4.94	8.00	0.633^{NS}	0.312^{NS}	0.688^{NS}	0.625	0.388	0.784

*5′ M13 tail: CACGACGTTGTAAAACGAC; F, forward sequence; R, reverse sequence; T_a, optimized annealing temperature; N_a, number of observed alleles per locus; N, number of individuals successfully genotyped; R, allelic richness; H_O, heterozygosity observed with P-values for the Hardy–Weinberg equilibrium test and significance threshold adjusted using the Bonferroni correction: P (5%) = 0.00152 corresponding to NS: >0.05; *$0.01 < P \leq 0.05$.; H_E, heterozygosity expected; Ba, Bader; Do, Dogona; Té, Téra.

gl-oe.asp). After testing all these primers, 11 microsatellite markers were kept and tested on 247 samples of *A. senegal* collected in three natural populations (Bader, Dogona and Téra) from Niger. Fragments were amplified by PCR with the multiplex kit QIAGEN® on a Mastercycler (Eppendorf) thermocycler in 10-μL reaction volume, according to the following duplex conditions.

Five microlitres of QIAGEN® multiplex mix, 0.08 μM of both forward primer with 5′-tail-end M13 (CACGACGTTGTAAAACGAC), 0.10 μM of both reverse primer, 0.10 μM IRDye fluorescent-labelled M13-primer (700 or 800 nm) and 5.0 ng of genomic template DNA. A touchdown cycling programme was used as follows: 95 °C for 15 min, 67 °C for 1.5 min, 72 °C for 1 min, followed by eight cycles of 94 °C for 30 s, 65 °C for 1.5 min with 2 °C decrease at each cycle, 72 °C for 1 min, 24 cycles at 94 °C for 30 s, 51 °C for 1.5 min, 72 °C for 1 min, and a final extension of 60 °C for 30 min. Amplified fragments were analysed at 700 and 800 nm by electrophoresis on an IR2-DNA analyzer (LI-COR 4200 sequencer) at the Montpellier Languedoc-Roussillon Genopole genotyping platform. Allele scoring was performed with SAGA software (LI-COR). Standard genetic diversity parameters were determined with FSTAT (Goudet 2001), which also performed the tests of Hardy–Weinberg equilibrium (HWE) and of linkage disequilibrium between pairs of loci.

The number of alleles per locus ranged from five (mAsCIRE10) to 25 (mAsCIRF02) (Table 1), and the mean number of alleles per locus was 10.9. Heterozygosity ranged from 0.016 (mAsCIRB09) to 0.852 (mAsCIRH01) in the Bader population, from 0.388 (mAsCIRH09) to 0.902 (mAsCIRF02) in the Dogona population, and from 0.044 (mAsCIRB09) to 0.784 (mAsCIRH09) in the Téra population (Table 1). Three pairs of loci (mAsCIRE08–mAsCIRE07, mAsCIRF03–mAsCIRB09 and mAsCIRB09–mAsCIRB10) showed significant linkage disequilibrium at the 5% level after Bonferroni correction. Only two loci (mAsCIRC07 and mAsCIRE10) in one population (Dogona) showed a significant deviation from HWE after Bonferroni correction (Table 1).The two other populations are in HWE. The observed departures from HWE can be interpreted as the result of a Wahlund effect or of the presence of null alleles or of both. Presence of null alleles was verified with MICRO-CHECKER 2.2.3 (van Oosterhout *et al*. 2004). Null alleles were suggested to occur over two loci (mAsCIRH09 and mAsCIRB09) in Téra and over four loci (mAsCIRE10, mAsCIRF02, mAsCIRH09 and mAsCIRC07) in Dogona; and no null allele was detected in Bader.

Microsatellite markers presented here were also tested using between four and 130 individuals of *Acacia laeta*, two *A. senegal* × *Acacia laeta* hybrids and 10 individuals of *A. senegal* var *leiorhachis*. Amplification products were obtained for all the loci in all species (Table 2). This result suggests that the markers described here can be used in genetic diversity studies on the *Aculeiferum* subgenus.

Microsatellites selected in this study constitute an efficient tool to investigate the genetic diversity and structure of *A. senegal* populations. They will be used to assess the mating system, gene flow, parentage and population dynamics of this species. Results will allow implementation of a strategy for its conservation and management.

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Table 2 Cross species amplification of microsatellite markers isolated from *Acacia senegal* in three other species: *Acacia laeta*, *A. senegal* var *leiorhachis* and hybrid between *A. senegal* × *A. laeta*

	Acacia laeta			*Acacia senegal* var *leiorhachis*			*Acacia senegal* × *Acacia laeta*		
Locus name	*N*	*A*	S_R	*N*	*A*	S_R	*N*	*A*	S_R
mAsCIRB09	126	5	303–341	10	4	297–305	2	2	299–327
mAsCIRB10	130	8	173–189	10	8	177–191	2	2	177–185
mAsCIRC07	4	4	152–168	10	5	152–168	2	2	160–162
mAsCIRE06	126	5	168–188	10	4	172–188	2	2	168–188
mAsCIRE07	4	3	154–160	10	5	148–160	2	1	154
mAsCIRE08	4	6	156–178	10	6	146–172	2	2	158–172
mAsCIRE10	4	2	117–123	10	3	117–123	2	2	117–123
mAsCIRF02	127	7	212–224	10	10	212–286	2	2	214–218
mAsCIRF03	4	3	274–334	10	7	274–336	2	2	274–328
mAsCIRH01	4	5	196–216	10	7	192–236	2	2	212–234
mAsCIRH09	127	6	135–156	10	4	135–150	2	2	135–141

N is the number of individuals that amplified, *A* is the number of alleles per locus, and S_R is the size range per locus.

References

Billotte N, Lagoda PJL, Risterucci AM, Baurens FC (1999) Microsatellite-enriched librairies: applied methodology for the development of SSR markers in tropical crops. *Fruits*, **54**, 277–288.

Chevallier MH, Brizard JP, Diallo I, Leblanc JM (1994) La diversité génétique dans le complexe *Acacia senegal*. *Bois et Forêts des Tropiques*, **240**, 5–12.

Chiveu CJ, Dangasuk OG, Omunyin ME, Wachira FN (2008) Genetic diversity in Kenyan populations of *Acacia senegal* (L.) willd revealed by combined RAPD and ISSR markers. *African Journal of Biotechnology*, **7**, 2333–2340.

Goudet J (2001) FSTAT *Version 2.9.3: A program to estimate and test gene diversities and fixation indices*. Available from http://www2.unil.ch/popgen/softwares/fstat.htm

Maslin BR (2003) *Proposed Name Changes in Acacia*. Australian Plants online available from http://asgap.org.au/APOL29/mar03-2.html

van Oosterhout C, 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.

Ross JH (1979) A conspectus of the african Acacia species. In: *Memoirs of the Botanical Survey of South Africa n°44* (ed. Killick DJB), p. 155. Botanical Research Institute, Pretoria.

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Isolation and characterization of 18 new polymorphic microsatellite loci for the swordfish, *Xiphias gladius*

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Abstract

Eighteen microsatellite loci (13 di- and 5 tri-repeats) were isolated from swordfish and characterized in two populations from the Atlantic Ocean and Mediterranean Sea. The number of alleles per locus ranged from two to 29 and the observed heterozygosity from 0.302 to 0.953. All but one locus conformed to Hardy–Weinberg expectations and there was no evidence for linkage disequilibrium between loci.

Keywords: genetic structure, microsatellite loci, swordfish, *Xiphias gladius*

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The swordfish (*Xiphias gladius*) is a highly migratory, large pelagic fish. It is found in the world oceans and the Mediterranean Sea, within a latitudinal range of approximately 60°N–45°S. It is of great commercial importance for world fisheries, and three inter-governmental organizations (ICCAT, IOTC and IATTC) are responsible for the management and conservation of this species in different oceans. The identification and delineation of stocks have become a priority and genetic markers can aid in defining population structure. The genetic structure of swordfish has been partly elucidated so far using mainly mitochondrial DNA markers (e.g. Alvarado Bremer *et al.* 1996).

In this study, we describe the characterization of 18 new polymorphic microsatellite loci in swordfish (Table 1). Two of the loci (VBC201 and XgSau98R1) were developed through cross-species amplification using primers designed from Gilthead Seabream (*Sparus aurata*) clones. Primers for locus VBC201 were designed from an EST-SSR clone of Seabream (AM961064) and tested in swordfish. Locus XgSau98R1 was initially amplified from a swordfish individual using the primers of locus SauH98INRA (AY173039; Launey *et al.* 2003), and then the polymerase chain reaction (PCR) product was cloned, sequenced and new specific primers for swordfish were designed.

Four of the loci were isolated from a genomic library screened for $(GT)_n$ microsatellite repeats using autoradiography, following the protocol described in Batargias *et al.* (1999). Twelve of the loci were developed by constructing an enriched genomic library following a modified enrichment protocol (Tsigenopoulos *et al.* 2003). Three micrograms of swordfish genomic DNA extracted from ethanol-preserved muscle tissue was digested with *Rsa*I (Minotech) and

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