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Cross-amplification and characterization of polymorphic microsatellite markers from *Acacia (Senegalia) mellifera* and *Acacia brevispica* to *Acacia senegal* (L.) Willd.

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Abstract

Seven polymorphic microsatellite markers isolated from *Acacia brevispica* and *Acacia mellifera* were successfully cross-amplified in *Acacia senegal*. The loci were surveyed for polymorphism using 30 samples. Allelic diversity ranged from 4 (*Ame02, Ab06 and Ab18*) to 13 (*Ab26*) per locus. The expected heterozygosity (H_E) ranged from 0.543 (*Ame02*) to 0.868 (*Ab26*) while observed heterozygosity (H_O) ranged from 0.516 (*Ame05*) to 0.800 (*Ame03*). Cross amplification of these loci represents a potential source of co-

dominant marker and will be useful in the study of genetic diversity, structure, gene flow and breeding systems of this important *Acacia* species.

1.0 Introduction

Acacia senegal (L) Willd. is a multipurpose agro-forestry tree, native to the arid and semi-arid lands of Sub-Saharan Africa but found as far as the Indian sub-continent. It grows to a height of 5-12 m, with a trunk up to 30 cm in diameter (Fagg & Allison 2004) and is valued for gum arabic production, as well as secondary agricultural roles, such as restoring soil fertility and providing fuel and fodder (Ballal *et al*. 2005). It is also essential in sand dune fixation and is preferred in bush-fallow rotation and intercropping (Anderson & Weiping 1992). The species has four varieties: *senegal*, *kerensis*, *rostrata* and *leiorhachis* but their delimitation is still problematic (Fagg & Allison 2004). Intraspecific variation has been reported in morphological, molecular and biochemical characteristics (Chikamai & Odera 2002; Motlagh *et al*. 2006; Al-Assaf *et al*. 2007). Despite its ecological and economic importance, little progress has been made in developing sets of molecular markers for genetic studies.

Microsatellites are among the best classes of markers for studying genetic processes in natural populations because they are abundant, co-dominant, interspersed throughout the genome, possess high resolution power and polymorphism (Chase *et al*. 1996). Their development, however, requires a significant investment (Fernandez *et al*. 2000); therefore, when markers have been developed in taxonomically related species, it is worthwhile testing for cross-amplification. In this brief preliminary study thirty-four

microsatellite primers developed from *A. mellifera* and *A. brevispica* were tested for cross-amplification in *A. senegal*.

2.0 Materials and Methods

2.1 Microsatellite loci screening

Genomic DNA was isolated from leaves following a modified CTAB procedure (Fernandez *et al*. 2000). Thirty four microsatellite loci were isolated from *Acacia mellifera* (Ruiz-Guajardo *et al*. 2007) and *Acacia brevispica* (Schnabel *et al*. 2005) were tested for amplification in 4 *A. senegal* DNA samples. PCR runs containing 20 ng of DNA, 1x PCR buffer (10 mM Tris-HCL (pH 8.3), 50 mM KCl), 2.5 mM $MgCl_2$, 200μ M of each dNTP, 0.1 μM of each primer, and 1 unit of *Taq* DNA polymerase (Invitrogen), were performed in a volume of 10 μl (Ruiz-Guajardo *et al*. 2007, Otero-Arnaiz *et al*. 2005). Reactions were carried out on an MJ Research thermal cycler following a touchdown protocol: 95°C for 3 minutes; 20 cycles of 95°C for 30 seconds, either 60 °C-50 °C, 58 °C-48 °C or 55 °C-45 °C (-0.5 °C) for 30 seconds, 72 °C for 30 seconds; 10 cycles of 95 °C for 30 seconds, 50 °C, 48 °C or 45 °C for 30 seconds, 72 °C for 30 seconds; final extension of 72 °C for 10 minutes and hold at 4 °C. PCR products were electrophoresed alongside 100 bp ladder, stained in ethidium bromide and visualized under UV-light. Loci that produced single, distinct bands at the expected size were selected for further screening.

Forward primers of the selected loci were tagged with M_{13} fluorescent tail and optimized using different annealing temperatures, PCR buffers, Mg^{2+}, dNTPs, primer and *Taq*

DNApolymerase concentrations. The mix that produced sharpest and most distinct bands at the expected size was selected (20 ng of genomic DNA, 1 X PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.05 μ M of each primer, and 1 unit of *Taq* DNA polymerase). These loci were then tested for polymorphism and reproducibility.

2.2 Polymorphism testing

The final set of loci was screened for polymorphism using a set of 30 DNA samples (3 samples per population from 10 populations). PCR was carried out as described above and products separated on 0.25 mm denaturing polyacrylamide gels containing 10% Ammonium Persulfate (APS), tetramethylethylenediamine (TEMED)-Omnipur and 8 % acrylamide (Gene-PAGE plus)-Amresco and electrophoresed using 1 X TBE buffer on a Li-Cor IR^2 4200 DNA sequencer. Band sizes were determined by referencing standard IRD800, 50-350 bp and 50-700 bp ladder (Khasa *et al*. 2005). Loci that were polymorphic and informative were then selected as a set of *A. senegal* markers for further studies.

3.0 Results

Out of the 34 loci screened, 11 were selected for polymorphism screening with a larger number of DNA samples while others failed to amplify or showed poor amplification (Table S1). During the screening, 7 loci (Table 1) showed positive amplification and unambiguous patterns of polymorphic bands and were selected as *A. senegal* markers. The loci showed features desired for co-dominant molecular marker in the species. No

deviation from Hardy Weinberg equilibrium or linkage disequilibrium was detected between loci (P>0.05) (Raymond and Rousset 1995). The number of alleles and expected heterozygosity were also estimated for each locus. The total number of alleles was highly variable across loci, ranging from 4 (*Ame02, Ab06 and Ab18*) to 13 (*Ab26*) (Table 1). Expected heterozygosity ranged from 0.543 (*Ame02*) to 0.868 (*Ab26*) (Table 1). Overall, the loci proved to be informative, at levels comparable to the source species and microsatellite loci of other tropical tree species.

4.0 Conclusion

This study unveiled evidence that cross-transferability of developed microsatellite loci can increase the availability of markers to address both ecological and evolutionary questions in *A. senegal*. These loci will form a useful marker set for population genetic studies of the species. These will include detecting and understanding available genetic diversity within the species range for maximum utilization and conservation purposes.

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Table S1: Microsatellite loci isolated for *A. brevispica* (Schnabel *et al*. 2005) and *A. mellifera* (Ruiz-Guajardo *et al*. 2007) used in cross-amplification study in *A. senegal.*

Locus Accession no.	Microsatellite repeat	Loci amplification
Ab01	$(AAG)_{15}$	Irregular amplification
Ab02	$(AC)_{11}$	Poor amplification
Ab03	$(AC)_{17}$	No amplification
Ab04	$(AC)_{7}$	No amplification
Ab05	$(AG)_{8}$ …	No amplification
Ab06	$(AC)_{9}(AG)_{10}$	Polymorphic amplification
Ab07	$(ATC)_{7}$	Irregular amplification
Ab09	$(AAAC)_{6}$	No amplification
Ab10	$(AG)_{13}$	No amplification
Ab12	$(AG)_{11}$	Irregular amplification
Ab13	$(AG)_{19}$	No amplification
Ab14	$(AG)_{10}$	Irregular amplification
Ab15	$(AC)_{10}$	Poor amplification
Ab16	$(AT)_{8}(AG)_{11}$	Irregular amplification
Ab17	$(AAG)_{14}(ATC)_{11}$	Poor amplification
Ab18	$(AAG)_{15}$	Polymorphic amplification
Ab19	$(AC)_{16}$	No amplification
Ab20	$(AG)_{24}(AC)_{16}$	No amplification
Ab21	$(AGAT)_{23}$	No amplification
Ab22	$(AG)_{11}$	Poor amplification
Ab23	$(AC)_{9}(AC)_{7}$	No amplification
Ab25	$(AC)_{7}$	Poor amplification
Ab26	$(AG)_{8}(AG)_{9}$	Polymorphic amplification
Ab27	$(AC)_{7}$	No amplification
Ame01	$(AAG)_{7}$	No amplification
Ame02	$(AC)_{7}(AG)_{11}$	Polymorphic amplification
Ame03	$(AG)_{9}$	Polymorphic amplification
Ame04	$(AG)_{10}$	Poor amplification
Ame05	$(ATC)_{7}$	Polymorphic amplification
Ame06	$(GT)_{10}$	Poor amplification
Ame07	$(GT)_{20}$	Polymorphic amplification
Ame08	$(GTT)_{9}$	No amplification
Ame09	$(GA)_{17}$	No amplification
Ame10	$(CA)_{9}$	No amplification
Ame12	$(CT)_{12}(AC)_{6}$	No amplification

S1- Supplementary material (Table 1)

Table 1: Microsatellite loci in *A. senegal* obtained from cross amplification from *A. mellifera* and *A. brevispica* including GenBank accession number; Ta - annealing temperature (touchdown range); bp - expected allelic size in base pairs; A - number of alleles observed per locus; n - number of individuals used; x, number of alleles recorded for the original species; H_O - observed heterozygosity; y, H_O recorded for the original species; H_E - expected heterozygosity; z, H_E recorded for the original species

Accession no.	Primer sequence	Repeat motif	Ta (°C)	Size (bp)	A (n) {x}	H_O {y}	H_E {z}
Ame02	F-GAACCATCAGCGTAATAA	$(AC)_7(AG)_{11}$	55-45	117	4 (30) {7}	0.670 {0.792}	0.543 {0.802}
DQ467674	R-GGTTTAGCAACATACTATCTC						
Ame03	F-GAACAATATCAGCAATCACT	$(AG)_9$	55-45	139	8 (30) {4}	0.800 {0.271}	0.810 {0.339}
DQ467673	R-CCTCATGCACACACAAGAT						
Ame05	F-CCCAACAAAGATCATCAT	$(ATC)_7$	58-48	203	5 (30) {13}	0.516 {0.532}	0.530 {0.580}
DQ467656	R-ATGGTTCAGTTTCTTTATTCT						
Ame07	F-ATAAAAACAAAAACCCAACTAAATG	$(GT)_{20}$	55-45	353	10 (30) {19}	0.695 {0.958}	0.802 {0.911}
DQ467658	R-GTCCAAAACTCTTCAATGTCAA						
Ab06	F-CCTTCTTTGACGGTATTC	$(AC)_9(AG)_{10}$	58-48	147	4 (30) {7}	0.785 {1.00}	0.745 {0.911}
AY843537	R-TCATCTCTCTTCTCCATT						
Ab18	F-GAAGGGTCTGGCATTAC	$(AAG)_{15}$	60-50	212	4 (30) {3}	0.717 {0.312}	0.579 {0.280}
AY843549	R-CGACGACGAAGATACT						
Ab26	F-ATATTCTGCTTTAGTCTA	$(AG)_8(AG)_9$	61-51	126	13 (30) {6}	0.780 {0.687}	0.868 {0.752}
AY843557	R-GGGGCATAAATATGAG						

* All the forward primers were tagged with M13R tag 5'-CACGACGTTGTAAAACGAC-3';