DEVELOPMENT OF MICROSATELLITE LOCI OF POD MAHOGANY,
Afzelia quanzensis (Fabaceae), by Illumina Shotgun Sequencing, and Cross-Amplification in A. africana

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• Premise of the study: Microsatellite loci were developed for *Afzelia quanzensis* (Fabaceae) as a first step toward investigating genetic diversity and population structure of the species in its native range.
• Methods and Results: Illumina shotgun sequencing was used to generate raw sequence reads, which were searched for potential microsatellite loci. A total of 70 potential microsatellite loci were tested for amplification and polymorphism, and 39 successfully amplified. Of the 39 loci that amplified, 12 were polymorphic while 27 were monomorphic. The 12 polymorphic loci were cross-amplified in *A. africana*, and eight successfully amplified.
• Conclusions: The 12 polymorphic microsatellite loci can be used for genetic studies of *A. quanzensis*, which can help determine its conservation status. Eight loci can also be used for genotyping in *A. africana*.

Key words: Afzelia africana; Afzelia quanzensis; Fabaceae; Illumina; microsatellite; PAL_FINDER.

Afzelia quanzensis Welw. (Fabaceae) is a deciduous, medium to large tree that naturally occurs in eastern and southern Africa. It is a lowland species that grows well in hot temperatures and sandy soils. Its wood possesses an ornamental grain, which is very strong and flexible. It glues firmly and takes a good varnish, properties that make it eagerly sought after by woodcarvers. Apart from woodcarving, *A. quanzensis* is also used for railway sleeper and door construction, and as timber for roofing and fencing. As a result, it has been heavily logged in its native range (Gerhardt and Todd, 2009). The International Union for Conservation of Nature (IUCN) has regionally listed *A. quanzensis* as vulnerable in Malawi (Golding, 2002), while in South Africa, it is now a protected species. No microsatellite loci have been developed specifically for the species. Here, we describe the development of microsatellite loci that will be used in genetic studies.

METHODS AND RESULTS

Genomic DNA was extracted from a leaf of one *A. quanzensis* individual (population geographic coordinates: 19°36.056'S, 32°30.084'E; representative voucher deposited at the National Herbarium and Botanic Garden, Harare, Zimbabwe [SRGH], voucher number 1) using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) following the manufacturer’s instructions. The DNA library was sequenced by an Illumina MiSeq Benchtop Sequencer (Illumina, San Diego, California, USA). The resulting raw Illumina paired-end sequencing reads were analyzed with a Perl script, PAL_FINDER_v0.02.04 (available at http://sourceforge.net/projects/palfinder), which identifies microsatellite loci without the need for prior sequence trimming and assembly (Castañol et al., 2012). The Perl script was run with Primer3 version 2.0.0 (Rozen and Skaltsky, 1999) for simultaneous primer design. Default settings were used except for the following adjustments: primer minimum annealing temperature (Tₐ) 50°C, primer maximum Tₐ 60°C, and primer optimum Tₐ 55°C. A total of 961,804 potential loci were identified, of which 7789 had primer pairs. We tested 70 potentially amplifiable loci with amplifiable primer pairs that occurred only once.

Of the 70 loci tested, 39 amplified successfully and these were checked for polymorphisms in 40 individuals randomly collected from a population near Chaseyama, southeastern Zimbabwe. Forward primers were tagged with a labeled Mi primer tail (TGTAAAACGACGGCCAGT). All PCR reactions were performed in a total volume of 10 μL, with 10 ng of template DNA, 0.6 μM of the reverse primer, 0.15 μM of the forward primer, 0.25 mM each dNTP, 0.6 μL bovine serum albumin (BSA; 10% w/v), 1 μL 10× reaction buffer with 15 mM MgCl₂, and 0.25 units of Taq DNA polymerase (Bulldog Bio, Rochester, New York, USA). Loci Afg45, Afg51, Afg62, Afg68, and Afg69 had an additional 0.1 mM MgCl₂. The thermocycling profile consisted of an initial denaturation at 94°C for 5 min; then 35 cycles of 94°C for 30 s, 55.0°C or 59.4°C (Table 1) for 30 s, 72°C for 30 s; and a final extension at 72°C for 7 min. The PCR amplicons were electrophoresed on an ABI 3730 DNA analyzer with GeneScan 500 LIZ (Applied Biosystems, Foster City, California, USA) as the size standard. The genotypes were scored using GeneMapper version 3.7 (Applied Biosystems).

Table 1 shows the 39 loci that amplified, their repeat motifs, number of alleles per locus, allele size range, and Tₐ. Twenty-seven loci were monomorphic while 12 were polymorphic. For the 12 polymorphic loci, number of alleles per locus (A), observed heterozygosity (Hₒ), and expected heterozygosity (Hₑ) were calculated using GenAlEx version 6.5 (Peakall and Smouse, 2006, 2012), and are shown in Table 2. The program Arlequin version 3.5 (Excoffier and Lischer, 2010) was used to perform further analysis.