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Development of SNP markers for Ayous (*Triplochiton scleroxylon* K. Schum) an economically important tree species from tropical West and Central Africa

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Received: 17 March 2016 / Accepted: 22 March 2016
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Abstract 182 SNP markers were developed for Ayous (*Triplochiton scleroxylon* K. Schum) by incorporating information from two next generation sequencing approaches (RADseq Floragenex and AFLPseq IonTorrent PGM) into a single genotyping panel for MassARRAY[®] iPLEX[™]. This set of markers was successfully used to genotype 753 individuals from 43 populations in five Tropical West and Central African Countries. These loci have an expected heterozygosity range of 0.007–0.501 and F_{ST} from 0 to 0.306.

Keywords Single nucleotide polymorphism · MassARRAY · Obeche · Timber tracking · Cameroon · Congo · Ghana

Introduction

Ayous (*Triplochiton scleroxylon* K. Schum), also known as Obeche, is an important timber species from the tropical West and central African forest, with approximately 38,000 m³ traded annually (ITTO 2015). The timber from Ayous is most commonly used in sauna panelling, house construction and table tennis rackets. It is found north of

the equator in monsoonal equatorial forests, with a discontinuous distribution from Sierra Leone eastwards through to Democratic Republic of the Congo and southwards to Gabon (Hall and Bada 1979; Igboanugo and Iversen 2004). Ayous is a large deciduous tree growing up to 50 m tall with a branchless trunk (bole) of up to 30 m. It is a pioneer species of primary forest, but also commonly found in secondary forests. Unlike other associated forest species, it has large distinctly lobed leaves (5–7 lobes, up to 20 cm across) (Hall and Bada 1979; Bosu and Krampah 2005; Orwa et al. 2009). Ayous is assumed to be self-sterile and outcrossing (Orwa et al. 2009).

Very little molecular marker investigation has been undertaken for natural populations of Ayous (Hardy et al. 2013). A previous study of the species by Akinagbe (2008) used Amplified Fragment Length Polymorphism's (AFLPs) only focused on Nigerian populations. Considering the importance of this species to the African economy, a detailed inventory of the genetic variation across its geographical range will be a powerful tool for forest monitoring and conservation and thus is considered a high priority. This paper presents a list of Single Nucleotide Polymorphic (SNP) makers suitable for such a purpose in Ayous. The SNPs were applied for the development of reference data to trace the geographic origin of Ayous timber, and thus could be used as a tool to enforce regulations to combat illegal logging (Degen and Henry 2015; Dormontt et al. 2015).

Electronic supplementary material The online version of this article (doi:10.1007/s12686-016-0529-8) contains supplementary material, which is available to authorized users.

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Marker development

SNP markers were developed by a reduced representation approach using either a restriction associated DNA sequencing (RADseq) protocol using two samples (see

Table S1) by Floragenex (Portland, Oregon, USA), hereafter referred to as the Thunen Institute for Forest Genetics (TIFG) approach (all P_ loci), or by the protocol of [Jardine et al. \(2015\)](#), using the two samples from the TIFG approach, along with an additional 46 samples (Table S1), hereafter referred to as the University of Adelaide (UA) approach (all A_ loci). DNA from all 48 samples was extracted by TIFG from cambium plugs or dried leaves using the DNA extraction protocol described in [Dumolin et al. \(1995\)](#). The TIFG approach was based on RADseq that combines genome reduction with a high coverage in the genomic regions analysed ([Baird et al. 2008](#); [Slavov et al. 2014](#)). Libraries were prepared using the restriction enzyme *SbfI*, and sequenced on the Illumina HiSeq 2000 platform to create paired end reads of 2×100 bp (Floragenex). SNPs were identified in the sequenced individuals using variant call format (VCF) 4.1 (Floragenex). For the UA approach, libraries were developed using the protocol of [Jardine et al. \(2015\)](#). Sequencing was done on the Ion Torrent PGM platform (Life Technologies) using the Ion Torrent PGM Sequencing 400 Kit. Sequencing read analysis was done using both the CLC-Genomic Workbench (Qiagen, Venlo, The Netherlands) and Geneious R6 (Biomatters, Auckland, New Zealand) programs.

Initial marker screening

Although undertaken separately, both marker development approaches used the following procedure for the initial marker screening. Ninety samples, consisting of the original 48 from the marker development stage, along with an additional 48 (Table S1), were used to screen an initial selection of markers. DNA of the extra samples was extracted separately for each approach; with TIFG using the [Dumolin et al. \(1995\)](#) protocol, and UA samples extracted at the Australian Genome Research Facility (AGRF, Adelaide, Australia) using the Nucleospin Plant II Kit (Nachery-Nagel, Düren, Germany). Suitable loci were identified in Assay Design Suite (ADS) (Agena Bioscience) and genotyped on the MassARRAY iPLEX platform (Agena Bioscience), using the iPLEX GOLD chemistry (Agena Bioscience). Genotyping was undertaken by either; INRA Genome Transcriptome Facility (GTF, Bordeaux, France) (for the TIFG approach) or by AGRF (for the UA approach).

Second marker screening

A second panel of markers was then compiled by combining successfully amplified markers from both development approaches into one panel using the ADS to design

the primers and multiplex groups. With this second panel, the genotyping of 911 individuals (Table S1) was undertaken, which included replicates of the 90 individuals used in the initial screening. DNA of the 911 samples was extracted at AGRF, from cambium tissue. Using Genodive ([Meirmans and Van Tienderen 2004](#)) and Genepop ([Rousset 2008](#)), tests for heterozygosity, global deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were undertaken. Monomorphic (or effectively monomorphic loci) were removed. Individuals and loci that had a <95 % genotyping coverage were also discarded from the final dataset.

Results

A combined total of 1667 variable SNP loci were identified as suitable for testing (see Table 1 for the breakdown per approach), of which 250 were incorporated into multiplex panels and tested for their screening suitability. A total of 238 loci were used to genotype 753 samples. Of these 238 loci, 56 were removed due to failure to amplify (42 loci), low representation (present in <95 % of individuals; 19 loci) and monomorphism (eight loci). A panel of 182 polymorphic loci is hereby presented (Table 2) along with their SNP allele calls, with forward and reverse MassARRAY sequencing primers. Expected heterozygosity (H_E) ranged from 0.007 to 0.501 and F_{ST} from 0 to 0.306. 65 loci were not in HWE and 57 loci were found to be significantly linked ($P < 0.005$) (Table 3). Genotyping runs between platforms showed >99 % concordance in allele calls.

This paper has identified 182 SNP makers that are variable for *Ayous* (*Triplochiton scleroxylon*) from across its geographical range in Tropical Africa and will allow for further genetic analysis to be undertaken.

Table 1 Comparison and number of loci found from either of the marker discovery approaches

Stage	Approach		Combined total
	TIFG	UA	
Marker development	1538	129	1667
Initial MassARRAY screening			
Used	133	117	250
Suitable	121	96	217
Second MassARRAY screening			
Used ^a	142 (9)	96	238
Suitable	108	74	182

^a Extra loci from the TIFG approach were included in the second screening. () denotes numbers

Table 2 Details of each SNP allele, with forward and reverse primer sequences

Locus	Alleles	Forward primer sequence	Reverse primer sequence
A_1018	A/T	CTTAGGAGAAAGGTAATGTG	AGTGAACCATGACATGGCTG
A_10244	C/T	GACTAATAATGCTCCAACC	GAACGTGAAATAGTCCCTGC
A_1056	C/T	CTAGGCTTGAAACGACCATC	GGAAGGAATGATACCTCCTG
A_1079	G/T	TATAGCATTTAGGGACCCAC	TCCCAAAGCTACTTATCCTC
A_1099	A/T	GCTGGCATTGACGTTGTAG	TTTCTTCTTGGAACTGCTTG
A_1109	C/T	CGTGGTCCCAGTATATATGC	GAACCATCGATAATCACATAC
A_1130_1	C/T	AATCAGTGTAAGTAGCTGCC	CCCTAGTTGAAAAAAGCAACC
A_11487	G/T	TTCGTCTTCTCTTTTCACC	GATCCACTCCATATTGAGGC
A_1167	A/G	TCCTTCTGAAGAAATTTGGG	AGCCAAGATTGAAATGGAAG
A_1194	C/T	TTAGTAAGGGGCTAAGTGGG	TATTGCAAATCAAGTAGCCG
A_1315	C/T	TGATGTGCCTTTTGAGGTG	GAACAACCAAGGCCAAAGAC
A_1521	A/G	CCATGTAGCAGCTGCATGAA	AATGTTGATTGTGGTGTTGC
A_15414	A/G	ACACCTTTTGGAGCGCTATG	ATCTCCTAGTTAGACACCTC
A_1625	G/T	CAGAGACTTGGACTTCAACC	ATTAGAGGAGTGGGTACAGC
A_168	C/T	TGGTCTTTGCACCTTTTGG	CAAGACTTCAAGCCATTGAG
A_1684	A/C	ATGCTTTCCTCCACATCAC	GTTAGGACTCAATGCAATGG
A_172	A/T	TTGGAATTGTGCTTGCATGG	CCAATGCCTTGATGATTGTG
A_1805	G/T	GTATGCCAAGTTTACATCCC	TTGAGGATGGAAAGAAGTGC
A_1900	C/G	CAACTTCAGCAGGATGGATG	GCCGTGTGAGAAAGATGTTG
A_1938	A/T	TTGATCCATAGAGACTAGAC	GCACCATTGCTTGAAACAAC
A_199	G/T	CCTTGTGTCAACTAAAACCC	ACCCTAAAGTGTAGCATCTC
A_208	A/C	CAAAGAACCAAACGTTACGG	AAAGCATGATGTCATGTCTC
A_23178	C/T	ATCAGTACACTTTACCACGC	GGAATGCAAGCACTAGCAAG
A_2440	G/T	AACGACAAGGAGCAAGAGAC	CAAGCAAGAATGGGATCTGG
A_2442	A/G	TTTATGGCCATCCTTCATGC	GTA AACCATAGAACCACC
A_253	A/G	GAAGATAAAGAATCAGGGTTG	TCATTGTCTGAACCTTACACC
A_25893	C/T	ATATGAACATGAGTGGTGGC	TTGTTGAGGTGCCATGCTAC
A_26	A/G	CGGAAAAAGATGATGGAGGG	TGGGAGAGAGTAGTACTAGG
A_2724_2	A/C	GCACCTTGATTTTCGGGTGAT	GGTTTGCAAGGACAAAGCTC
A_2753	C/T	TGAAGCCTTAGCCATTTCTC	GTGAGTCTAAAATAAGCGTC
A_2841	G/T	AATTTGCTGGCTGCCATCAC	GAATTCATATCAGACGTTT
A_2942_1	C/T	GAACCAACAAGCCAGCAAAAG	GCTACAAAGAACACTCTAAG
A_2942_2	A/G	TCATCGACATAAGACCAGAC	GGCTTGTTGGTTACAGTTG
A_31129	G/T	ACATCCCATCATTGAAGCCC	GGATGCAAAAAGGCATGAAGC
A_3189	A/T	GTATCACCAGAATGACTAGC	CATAAGCTTTGTGCAGCCTC
A_3628_1	G/T	AGACAAATTTTCCACAAAG	TGATGGGTCTATACTATGGC
A_368	C/T	CCAAATGCACAAACTCTGGC	GAAGTTCTTTGCCAAGGCTC
A_387	G/T	CCATTTGCCAGCTTGATCAC	TCCCTAGTCTTGCTACTAAG
A_389	A/C	GTCTTCTGGTTTCACATCCC	GGGCTGTAGAAGCAGAAAAC
A_4_1	C/T	AACTCGGCCGGTACTTCAC	TGACCAACCTCGTAAGTCTG
A_4_2	A/T	ACAAGAGAGTTGGTTGAGGC	GACAGGAAAACCTCCCGTTG
A_4037	C/T	CAGAACAACCTCCATGACAC	AACTAGGCAACATGAAAGGG
A_407	A/G	GGTAATCTTGACCATAGGAG	GCTCGACTTATTGCTAAGGG
A_411	A/G	TGCTAGGACCATACTTGGTG	TTCAAATGTGAAGCAATGGG
A_412_1	A/C	GTATTTGTATTACATTCTCC	CCTAGCTTATGATGCCAAAG
A_412_2	C/T	TTCTTCTGTAAATCATCAC	ACAAGGGTAAATGTCTAGC
A_4249	A/C	GGACAGCAAGCATAAAATGG	CTTGACAGCAAAATTGCCAG
A_435	A/C	CTATGTTACATTACATGGC	ATCCAATAGGTCTCAGACAC
A_444	A/T	TCCTCGAATTCAGGAGGAAC	ATGAGGTTGATGGAGGAACC
A_532	C/T	CGATGTTTCTGTTCACACAC	CAGAGTCAGTTTGGTTACAGC

Table 2 continued

Locus	Alleles	Forward primer sequence	Reverse primer sequence
A_5394	A/T	CTCGATAGGACATGAGATGG	ACACGGCACGAATTCAAAGC
A_55	A/C	TGACAATCGAGCATTGCAAG	CTTATGCACAAACGTCTCC
A_55761	A/G	GAAAGAGCTTGTAAGTCAAGT	CTGCTTACATTTTGATGCTG
A_6020	C/T	TTAGGTATCTACACCGCCAG	TATCCACGATGGTGTATGGG
A_626	A/C	TGAAGGTGCCTCTGTTTATG	CCCACTCAAACCTCTCTTCAC
A_642_1	A/C	TGATAAAGCTTTGGATCCTC	GGAACGAATTGCTCATCCAG
A_642_2	A/T	TACATTGTTTGGCGATGAGG	GGAGTTGGCTTCCCTTCATC
A_6645_1	G/T	CATTACCTCACCATCCTCAC	GTGCTTCATTGCAAAGGG
A_6645_2	A/G	GTACTTGGTAGGCATCATTTTC	AGCAACACGATTATATCCG
A_665	A/G	TTAGACACCTCCACTTGACC	TGTACTCTTCCAAAATGCAC
A_698	C/T	ACAGTAGGTGTAGATGCAAC	GGTGTACAAAATTACATGGG
A_71422	C/T	ATGAGATTGACTAAGCTGCC	GGTTTATCCACTTGATGGC
A_73919	A/G	AAGCAGGGATCGCAACATTC	TCAGATTTGTGACCAGCGAG
A_77365	A/T	TGTA AAAAGGCATTGCCTTAG	GAAGCCAAATCATGAGATGC
A_827	A/T	CTTCAACTTTGTATTGCCTC	CCGTTCTCAAGACCTTCTAC
A_8898	A/G	TGCGATACTCCTAGGGAATC	TCATGAGTCTCGCTACAAG
A_913	A/C	CCTACTCAACCTATCATCGC	GAAGTATAAGCGTGTGAGTC
A_929	G/T	CTTGGCAAGCGATCTATGAG	GTCCATCTGCGGTTCCAATC
A_935	C/G	AGCCACTATCTCACCTTAG	TTGATGGATCTGCTTACGGG
A_9516_1	C/G	TCCTTATCCTCCTTCTGATG	TGGACAGTGGAAAGAAATCG
A_9516_2	C/T	CACTGCAGCTGTCATCTATG	ATCAGAAGGAGGATAAGGAG
A_961	C/G	TTATAGCCTAACGAGGTCGG	TAACATCTTGCCACGTCGTC
A_CS_110	A/G	AAGCAGGGATCGCAACATTC	TCAGATTTGTGACCAGCGAG
A_CS_165_1	C/T	CTTGCCTTCATTCTCCTGC	GGAAAATATGGGTTTGAAGC
P0065	A/G	GGATGACTTGTTTGATGTGC	AAATCTGGTCCCTCAGCAAG
P0112	A/T	GAGTAACAGAGTGTTGCTCA	TACAATTTGGGAGAATGGAG
P0133	C/T	GGTGGAAAGCAAACAAGGAG	TGCCACCTATAGCAATGCAG
P0182	A/G	ACTGGGTTGACTCCAGATAG	GACAATATCAAGTAGTAGGG
P0245	A/C	TCACCCTGCTCAAGTCATTC	TATGTGTGCGTCTTTTCGG
P0265	C/T	TCCTATCAGCATTTCCACTC	TGCAGATAAGGTGGCAAAGG
P0380	C/T	TGAAGCAAGCACAAGACAGG	TGCTCCTGCATTTGTTCTCG
P0616	C/T	TACGAAGAATAAATAAGAAG	CCTCTGGATACTTAGCTTCG
P0761	A/T	GTCTTTCCAAGCATTCTCC	GACAGGAGTACCATAAATC
P0785	A/G	GATGCGGATATCTGCTCTTG	AAAACCAAGACTGCACACCC
P0809	A/G	GCCACCTTCTTTGCTATCAG	AAACCTTCTTCGAAGCCCTG
P0812	C/T	AGGGAGTAGAGACTAAGAAC	GTGCACACATTTGATTTGCG
P0855	G/T	GGCGAGAATAGAATTAATG	TCATTACAAGAGCTGGGAGG
P0884	G/T	TTGAAGGAGGCCATTCTAC	CATATACATCGCGTCTCCTG
P0896	C/G	GGGAGGTTTCATGTTGTTTAC	ATTATGATGAGGGTTTCATCG
P0917	A/G	GCAAGATGAGGACGATGAAG	TCTTCGTCGTCTTGATTCCG
P0981	C/T	TAGTGTCTTAAGAGGATCAC	GCTTTGGGTTTGAACATACC
P1064	A/G	CCAGTTTGCACAACACCTTC	CCAACACATACCTTTCATCG
P1094	C/T	AAATGTCTCGAGCTTCAGGC	CAATGCAAGTCCCATAACC
P1103	C/T	GGGTACTTTACAAAATGAC	TTCTGGCGAATTCTAAGCAC
P1165	A/T	TTCTTTCTGTTGTCTGGGTG	AGAATGACCGCATTCCCTTC
P1265	C/G	ATTCCCGCAACGGAATTAC	CTTCCATGTAGCTGGCTTC
P1477	G/T	CCAACCACAGCTTCTATTTC	TTTGCTGACCTTGATCCCAC
P1481	C/G	CGGGCTGATCTATTTCGAAC	ATGATGCAGAACCTATAGAG
P1547	C/T	TCTCCTTCTTTGATGGTGAG	GAGACTGCATCAGTTATGGC

Table 2 continued

Locus	Alleles	Forward primer sequence	Reverse primer sequence
P1559	A/G	AGCTTGAGCATTGCTAGGG	TTAGATTGCTGATCACTCGG
P1813	A/T	TTAGTGCATATTTGCTCGTC	TTGAGAGAGAAAAGAGAGAG
P1835	C/G	CTACTGATAGAAGCCATAGG	TGAGAATGGTTGTGTTACTG
P1860	C/T	CATCATAGACAGCTTGACCC	AACATAGGATTCGGCCCAAC
P1894	C/T	ATATTAGAGGGCTAGGCCAC	TTTGGTTGGAACCTAAGGGC
P1918	C/T	TGTTTCAGATTATTGTGCC	TCAGCAACAGCAGGAAGTAG
P1919	C/G	AGAGGCCTTCGATAAGATGG	CGTAAGGTTAGTGCTTACAG
P1960	C/G	GGTGCAAATTTCTACCCCTCC	AGCCTATATATTGCAGCCAC
P2146	A/C	TTGGACTTTCCTTAGGCTTC	GCCGAAAAGTTGAACTGGAC
P2274	C/T	GGTCATGCTAAGTGTAAGTGG	CAGGCCATGTCAGTGTAATC
P2290	A/G	CTTCAGAGGATGAGAAACAG	CTACCTTGATGAAGCAAGTC
P2328	C/G	ACATGCATGAAATCATAGGG	GGTGGATGAAGCTTTTCACG
P2367	A/G	GACCAGTGAATCTAATTGGC	CTGTTCTTCTCAGAGGGAG
P2464	A/G	CACGGTAATCATGGGATTGG	CCCTCTCTGACATAAATAG
P2496	A/G	AATGCCGATGGAATGGGAAG	ACTTGAGCAGTTAAAACTCC
P2618	A/T	TTCGGTGCAAGGAAATAGGG	ACTTTGCCAACTCCAAGCAG
P2644	A/G	ACATTGCCACTAAACCACCC	GAATAGGACAGCTACACTGG
P2679	C/T	AACGAAAGGGCAAAGCTCC	TTGGTCTCTTTTCATCTGC
P2722	A/T	CCTTTTCAGTCTTCTTACC	TTTCTTTTTCAGGTCCGC
P2733	A/T	CCCTATAATTTCCATTGCC	AAGCACCTAGTTAGCTTTCC
P2749	C/T	ATGAGGAGTGGGTGAGAAAC	GATCTGTCATAATTCCGAGG
P2775	C/T	GAGAATTCCTCCTCTTGACC	GGGTCAAACCCACACTTAC
P2967	A/T	AGCATGATACTTGTCTACCG	CATCCATGAAGTCAAGTGCG
P3076	C/T	TCAGGACTGGTTGATGAAGG	CGCCCTAGTAGGTCAACCAT
P3093	C/T	CCTGCAAACCGGTGATTTTC	CATCGGTTATTGATGCCTGC
P3137	A/T	TTACCTGCACGATATGTCCC	GTCAGTTTCTCCTGAGTTGG
P3285	A/G	CTTATGTCATTTCCATCGGG	AGGGTCTGATCATGAATGGG
P3303	C/T	CTCTATCTCGTACCTAGAAC	ATCAGCGCCAATGCCAAAAC
P3414	C/G	CCGAGACAGGATAGTCAAAG	TCAAACCACGGGCAAGAAG
P3480	C/T	TCCAAACAAGGAGGATGCTG	TACAGCTAGTTGGACTCCAG
P3657	A/G	AACAACCATTGCCATGACGG	AGCAGTCATGATGCAAACCC
P3699	A/G	TCAACCTCTTCTCCTCTTC	AAGGGTTTGGTATCATCATC
P3722	C/T	GGCACGTGCTTTTTGCCTAT	GTGTCCTTTCCCTCTACTC
P3752	C/T	CCCCTCAAGCTTGATATTCC	ACGGACGAGGGAAGCATTAA
P3788	C/T	TCCTCTGTAAGGTATGTCCC	CTGAAAACGCAGGCGAATAC
P3997	C/G	GTAGTAGGCTTATTAACGAC	TCTTATCCTTCTCAGAGCCC
P4042	G/T	TTGATGAAGTGGTCAGCTTG	CATGCTTACGCAAGTAAACC
P4293	A/C	TGTTGCTCAGCAATTGCAGG	GAAACCTCGAAGGCAATCTC
P4294	C/T	ACTGCACATTCTAGTGGAGG	GCATATGAGATCCACTTTCC
P4492	A/G	TCTCAGCTGCAACAAGCAAC	CCTTCTCGGCTATTCTGTG
P4617	A/G	AGACCAACCAAAGGAAGCTG	GTGGGTGAATGAGTGGAATG
P4629	A/G	CTCCAATTCTCGAAGTCAAC	AGCATTGCTGTCCAAGTTG
P4638	C/G	ACTATTTGCATGCTGCAGGG	TGAGTTACAACCTCCTCCTC
P4706	A/C	ATGAAGTCCCTGGCCATTTC	GGCATATGGTTTTTGTGCTT
P4772	A/G	CTCTTTGAGCTAATCACAGG	ACCTTTGCACTTAACCGGTC
P4837	A/G	CTGCACACTGGATGCATTAG	CAGTTTGGATCGGCTTCAAG
P4872	C/T	CCACATGCTGGGTTTGATTC	TGATCCCCATTTTAGCTTG
P4926	C/T	GGTTTTATGCCTAGCTACAC	TGCTCTTATAGTCTTTTGCC
P4928	A/C	TTGTTCCGTGCTTCGTGATG	CCAAAAGCCCATTTCACTAC

Table 2 continued

Locus	Alleles	Forward primer sequence	Reverse primer sequence
P5178	A/G	CCGTCTATTCATATTCGCTC	AAAATCATGGCACGGATAAG
P5240	C/T	TGGATCGAGCCTGCAATTGG	AGGTGGTTCAGCTTGAAATC
P5264	A/T	TCAGTGTGATTGGTTAGAGG	CATCTTCAGTCAGAGGAAGC
P5404	G/T	CTGAGCTCAAGTTAGAAGCC	TGTGGGAATTCATGGATAC
P5439	G/T	CTACAGCTAGAATATGACCG	ATTAGTCCGTATGTGACGCC
P5462	C/G	GTGCATCTTTGTGTTGACTC	ATCTCTTCCTTGCTGGAGTC
P5532	A/C	CTGTGTTTCTTTCCCTTTC	TGCTAGAAAACACTACGAAGCC
P5562	C/T	CGGTCAAGGTCATGATCAAG	GAGCCAATTTGGAAAATCCG
P5587	A/G	CTAAATCTCATGAGGACGGG	TTTTGGAGGACTGTAATCTG
P5700	C/T	TGATGCTTCTCACTCTGTTC	CCAGCATCGACATTTGACAG
P5715	A/G	AACAAAGCAAATGACACCTC	ATCGAGTCTGAAAATCTGTC
P5737	C/G	CTTGACGCAACAAAGCGCAC	ATGATCAAATCAATCCCGGC
P5777	G/T	TAAATCGATCGGGTCTGTC	CTCAAAACACAGCCTGGTTC
P5888	A/G	ACTTGACCTCTTCTGGCTAC	GGGAGTATGGTCTATGTAAG
P5909	G/T	TCCTCCAGAAGGGAAAATGC	CAACCATGAAGCCTACACTG
P5944	A/G	GCAGTAGCAGACCAAGAAAAC	GGTTCGGTCATCTTGAAAAGG
P5972	A/G	CAAACCAAAAAGCTTTCAGCAG	CAGACTATTCTGATGCATGCT
P6137	A/G	ACTACCTAGAGAGATCAAGC	TGAAGTTCATGTCCAACCTG
P6163	A/G	CTCTGTTCCAGGATGAGTTG	GCCCCACAACATATATCAGG
P6225	A/G	CAAGAGACTTACCATAAGCC	GACCCTTTGCTATGAAATGG
P6238	A/C	TTATTTGGGTGCGGGATCGG	TTGTCATCAGCAGCCTCTTC
P6277	A/G	GCAACTCTATACTGATAGAGG	ACAACGCCAAATACACATGC
P6290	C/G	GACCCTAGCTAACATCGAAG	GAGTCAGAACCAAAGGAAGG
P6328	C/G	GTACTTCCCAACCTCTACAC	TGGTCATAGACCATCAGTGC
P6392	A/G	GCATGCATATTTTTCAAAC	CCTAAACAGAGAGGGAAGAC
P6399	A/G	TTGCTTCACTAAACTCCCCC	TAGTCATTGTCAAGTTGGGTG
P6402	A/T	AATATCCCGGTTTGAACCTCG	CAACTACTCCATTGACAGGG
P6483	G/T	ATGGATTCAACTGAGATGTG	AAGACTGACTGGACAGTGGC
P6527	C/T	TGAGCCACAGTTGATACCAC	AGAAAGCGGCTATCCTAGAC
P6533	C/T	CCTGTCCCAACCATTTTGTC	CCAGTCTCTTTCTCCTTTC
P6618	A/G	CGGAACAACAATATTGTCTC	ACATCGATCTTGGGAGGTTC
P6704	G/T	GGAGCAGGAGGAATTTAAGG	GGTTCATGTAACAAGGTGGC
P6741	C/G	TGGAGTCCTTGAAACTAGAG	ACATTTCCCATCCAGGAAGC
P6787	A/G	TGCAGGTAATCTGGACTTTC	TTTGCAACAGTCATGGCTGG

Table 3 Population genetic parameters of markers developed in this study

Locus	H _O	H _E	F _{ST}	HWE	Linked loci
A_1018	0.093033	0.091	0.018	Yes	
A_10244	0.517756	0.472	0.017	No*	
A_1056	0.052314	0.052	0.031	Yes	
A_1079	0.027012	0.058	0.017	No****	
A_1099	0.401666	0.383	0.044	No*	A_626
A_1109	0.144287	0.165	0.011	No**	
A_1130_1	0.040127	0.138	0.063	No****	
A_11487	0.113827	0.11	0.132	Yes	A_2841
A_1167	0.063241	0.063	0.007	Yes	A_1684
A_1194	0.03706	0.039	0.011	No*	
A_1315	0.019547	0.033	0.028	No****	
A_1521	0.172077	0.179	0.031	Yes	
A_15414	0.097745	0.344	0.064	No****	
A_1625	0.156959	0.157	0.024	Yes	
A_168	0.048916	0.053	0.035	Yes	
A_1684	0.103366	0.101	0.032	Yes	A_1167
A_172	0.012662	0.015	0.018	No*	
A_1805	0.0283	0.05	0.016	No****	
A_1900	0.203873	0.182	0.068	No*	A_4249
A_1938	0.174097	0.328	0.092	No****	
A_199	0.160011	0.178	0.138	No***	A_6645_1, P1477
A_208	0.055877	0.06	0.027	Yes	
A_23178	0.429399	0.438	0.07	Yes	
A_2440	0.071372	0.074	0.015	Yes	
A_2442	0.488105	0.409	0.051	No****	
A_253	0.355546	0.378	0.073	Yes	
A_25893	0.170731	0.215	0.051	No****	
A_26	0.173093	0.154	0.057	Yes	P3093, P6402
A_2724_2	0.501239	0.466	0.031	Yes	
A_2753	0.330884	0.302	0.165	Yes	A_77365, P5737
A_2841	0.136742	0.275	0.159	No****	A_11487
A_2942_1	0.537573	0.501	0.014	Yes	A_2942_2, P0380
A_2942_2	0.228849	0.336	0.046	No****	A_2942_1
A_31129	0.027852	0.04	0.007	No****	
A_3189	0.283377	0.248	0.059	No*	
A_3628_1	0.028755	0.029	0.01	Yes	
A_368	0.085982	0.085	0.008	Yes	
A_387	0.087368	0.123	0.039	No****	
A_389	0.064079	0.063	0.001	No****	
A_4_1	0.410845	0.426	0.021	Yes	A_4_2, A_532, P0785, P5404
A_4_2	0.149808	0.144	0.01	Yes	A_4_1, P0785
A_4037	0.450415	0.474	0.018	Yes	P3657
A_407	0.489102	0.46	0.082	Yes	
A_411	0.24864	0.24	0.08	Yes	
A_412_1	0.2681	0.232	0.138	No*	A_412_2
A_412_2	0.247073	0.221	0.021	No**	A_412_1, P3137
A_4249	0.2951	0.371	0.096	No****	A_1900
A_435	0.153395	0.152	0.03	Yes	
A_444	0.442758	0.458	0.036	Yes	A_532, A_77365, P0182, P0761, P0917, P4837

Table 3 continued

Locus	H _O	H _E	F _{ST}	HWE	Linked loci
A_532	0.038873	0.031	0.185	No****	A_4_1, A_444, P1103, P3699, P5404
A_5394	0.230543	0.242	0.062	Yes	
A_55	0.152034	0.165	0.022	Yes	
A_55761	0.198766	0.225	0.06	No*	
A_6020	0.144222	0.171	0.018	No**	
A_626	0.155507	0.14	0.141	Yes	A_1099
A_642_1	0.328028	0.326	0.306	Yes	
A_642_2	0.010982	0.011	-0.001	No****	
A_6645_1	0.26864	0.259	0.024	Yes	A_199, A_6645_2, P5264
A_6645_2	0.482689	0.479	0.027	Yes	A_6645_1
A_665	0.238999	0.218	0.032	Yes	P3303
A_698	0.472466	0.431	0.003	Yes	P6483
A_71422	0.020076	0.022	0.008	Yes	
A_73919	0.210979	0.215	0.045	Yes	A_CS_110
A_77365	0.460907	0.458	0.052	Yes	A_2753, A_444, P4837, P5737
A_827	0.397321	0.404	0.031	Yes	
A_8898	0.477334	0.469	0.034	Yes	
A_913	0.083644	0.118	0.028	No****	P6527
A_929	0.107053	0.113	0.036	Yes	
A_935	0.361777	0.364	0.03	Yes	
A_9516_1	0.397059	0.414	0.084	Yes	
A_9516_2	0.176745	0.181	0.031	Yes	
A_961	0.041062	0.041	-0.005	Yes	
A_CS_110	0.209318	0.214	0.046	Yes	A_73919
A_CS_165_1	0.146411	0.151	0.028	Yes	
P0065	0.060671	0.065	0.01	No*	
P0112	0.34828	0.334	0.058	Yes	
P0133	0.345986	0.338	0.091	Yes	
P0182	0.51621	0.496	0.001	Yes	A_444, P2749
P0245	0.0211	0.021	0.008	Yes	
P0265	0.351575	0.372	0.047	Yes	
P0380	0.272244	0.259	0.128	Yes	A_2942_1
P0616	0.249607	0.253	0.048	Yes	
P0761	0.500303	0.48	0.03	Yes	A_444, P4617
P0785	0.43884	0.474	0.022	Yes	A_4_1, A_4_2
P0809	0.166041	0.182	0.025	No*	
P0812	0.378764	0.414	0.101	No*	
P0855	0.019989	0.019	0.059	Yes	
P0884	0.045946	0.048	0.02	No*	
P0896	0.396963	0.393	0.009	Yes	P1094
P0917	0.266164	0.244	0.016	Yes	A_444, P4837
P0981	0.317668	0.35	0.039	No*	
P1064	0.014831	0.016	0.028	Yes	
P1094	0.332687	0.336	0.012	Yes	P0896
P1103	0.492627	0.485	0.023	Yes	A_532
P1165	0.211522	0.215	0.058	Yes	
P1265	0.384077	0.384	0.039	Yes	
P1477	0.206337	0.183	0.083	Yes	A_199
P1481	0.009252	0.009	0.06	No****	

Table 3 continued

Locus	H _O	H _E	F _{ST}	HWE	Linked loci
P1547	0.072262	0.07	0.014	Yes	
P1559	0.346004	0.351	0.202	Yes	
P1813	0.026355	0.025	0.036	No****	
P1835	0.016081	0.015	0.031	No****	
P1860	0.301467	0.3	0.268	Yes	
P1894	0.338365	0.361	0.034	Yes	
P1918	0.08072	0.083	0.049	Yes	
P1919	0.333867	0.455	0.003	No****	
P1960	0.475925	0.446	0.047	Yes	
P2146	0.178643	0.261	0.072	No****	
P2274	0.214375	0.252	0.02	No*	
P2290	0.361529	0.34	0.111	Yes	
P2328	0.091182	0.091	0.039	Yes	
P2367	0.447908	0.425	0.045	Yes	
P2464	0.345378	0.357	0.064	Yes	
P2496	0.099038	0.104	0.045	Yes	
P2618	0.265158	0.41	0.132	No****	
P2644	0.51302	0.49	0.005	Yes	P5737
P2679	0.276638	0.322	0.113	No**	
P2722	0.09604	0.103	0.077	Yes	
P2733	0.279262	0.332	0.037	No***	
P2749	0.441156	0.442	0.105	Yes	P0182
P2775	0.371264	0.381	0.034	Yes	
P2967	0.192119	0.197	0.042	Yes	
P3076	0.446435	0.431	0.027	Yes	P3093
P3093	0.447167	0.442	0.053	Yes	A_26, P3076, P6402
P3137	0.281166	0.264	0.065	Yes	A_412_2
P3285	0.089765	0.091	0.013	Yes	
P3303	0.339193	0.374	0.038	No*	A_665
P3414	0.301193	0.322	0.104	Yes	
P3480	0.074833	0.089	0.052	No**	
P3657	0.448743	0.451	0.048	Yes	A_4037
P3699	0.444768	0.44	0.105	Yes	A_532, P6533
P3722	0.092981	0.111	0.027	No*	
P3752	0.263187	0.311	0.106	No****	
P3788	0.036137	0.035	0.021	No****	
P3997	0.395838	0.393	0.006	Yes	P6483
P4042	0.235358	0.367	0.194	No****	
P4293	0.071199	0.08	0.037	No**	P6328
P4294	0.064661	0.069	0.002	No*	
P4492	0.151633	0.152	0.04	Yes	
P4617	0.234324	0.242	0.028	Yes	P0761
P4629	0.093062	0.093	0.016	Yes	
P4638	0.475152	0.479	0.035	Yes	
P4706	0.519359	0.48	0.027	Yes	
P4772	0.114793	0.137	0.008	No**	
P4837	0.446817	0.45	0.007	Yes	A_444, A_77365, P0917
P4872	0.204229	0.231	0.039	No****	
P4926	0.08712	0.098	0.049	No*	

Table 3 continued

Locus	H_O	H_E	F_{ST}	HWE	Linked loci
P4928	0.014569	0.014	0.02	No****	
P5178	0.004546	0.007	0.007	No*	
P5240	0.294353	0.278	0.06	Yes	
P5264	0.399463	0.391	0.074	Yes	A_6645_1
P5404	0.293849	0.295	0.15	Yes	A_4_1, A_532
P5439	0.018491	0.018	0.033	Yes	
P5462	0.460152	0.478	0.014	Yes	
P5532	0.339573	0.359	0.04	Yes	
P5562	0.373921	0.399	0.09	Yes	
P5587	0.226824	0.236	0.027	Yes	
P5700	0.103751	0.141	0.006	No****	
P5715	0.022219	0.021	0.055	No****	
P5737	0.40955	0.486	0.022	No****	A_2753, A_77365, P2644
P5777	0.409202	0.411	0.117	Yes	
P5888	0.09932	0.097	0.014	Yes	
P5909	0.006722	0.007	0.029	No****	
P5944	0.17922	0.185	0.044	Yes	
P5972	0.169764	0.172	0.032	Yes	
P6137	0.01898	0.018	0.031	No****	
P6163	0.129667	0.124	0.018	Yes	
P6225	0.416036	0.401	0.141	Yes	
P6238	0.337492	0.325	0.156	Yes	P4293
P6277	0.067269	0.07	0.073	Yes	
P6290	0.438493	0.462	0.018	Yes	
P6328	0.007559	0.008	0.016	No****	
P6392	0.33798	0.337	0.251	Yes	
P6399	0.049765	0.071	0.097	No***	
P6402	0.332317	0.459	0.048	No****	A_26, P3093
P6483	0.297177	0.283	0.097	Yes	A_698, P3997
P6527	0.367753	0.342	0.022	Yes	A_913
P6533	0.45612	0.439	0.053	Yes	P3699
P6618	0.477722	0.452	0.072	No**	
P6704	0.183277	0.201	0.041	Yes	
P6741	0.331152	0.318	0.024	Yes	
P6787	0.481235	0.461	0.008	Yes	

H_O observed heterozygosity, H_E expected heterozygosity, F_{ST} infinite allele model, HWE Hardy–Weinberg equilibrium

Asterisks refer to significance of deviation from HWE, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

Acknowledgments The work was financially supported by the International Tropical Timber Organization (Project PD 620/11 Rev.1) and the Australian Research Council (Project LP120100648). The TIFG approach for loci screening was supported by funding from the Conseil Regional d'Aquitaine (Nos. 20030304002FA and 20040305003FA), European Union, FEDER (No. 2003227) and from Investissements d'avenir, Convention attributive d'aide (No. ANR-10-EQPX-16-01).

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