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Short communication

Development and characterization of microsatellite markers for *Osyris lanceolata* Hochst. & Steud., an endangered African sandalwood tree species

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Osyris lanceolata Hochst. & Steud. is a multipurpose tree species widely spread in many of the sub-Saharan countries ranging from Algeria to Ethiopia all the way to South Africa. In Kenya, the species is endemic to the Arid and Semi-Arid Lands (ASALs). It is highly valued for its essential oils used in the cosmetic and pharmaceutical industries. Despite its endangered status and economic importance, little is known about its genetic diversity status and only few conservation strategies exist for the species. Overexploitation of the species has resulted in the decline of its population and reduced availability of its products. The mode of harvesting of sandalwood is destructive and unsustainable. This is because the whole tree is usually uprooted to get the heartwood from the stem, stump and roots. The exploitation of African sandalwood could soon drive the species to extinction unless proper control measures are put in place through regulation of its trade and development of conservation strategies. Despite its endangered status and economic importance, no genetic study has been carried out on the species to provide information vital for conservation strategies. This paper reports the development and characterization of a set of 12 polymorphic and five (5) monomorphic microsatellite markers isolated and characterized of *O. lanceolata*.

One plant leaf sample was used as the source of DNA for genomic library construction. Total genomic DNA was extracted from silica gel dried leaf using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The DNA sample was then sent to The Gene Pool Institute of Evolutionary Biology, University of Edinburgh for sequencing. Simple sequence repeats (SSRs) were extracted through PAL Finder software version 0.02.04 (Castoe *et al.* 2012) and primer pairs developed. Identified microsatellites and designed primers were assembled using QDD (Meglécz *et al.* 2010) with parameters given in set_qdd_default.ini.file. The gaps emerging during the scaffolding process were closed using GapCloser (vs. 1.12). The contigs >1000 bp of the draft assembly were analyzed and functionally annotated using Blast2GO (Conesa *et al.* 2005). Based on this information, 48 primer pairs consisting of either di- or trinucleotide repeats were selected. After testing, 17 primer pairs were identified and used to characterize 84 samples of *O. lanceolata* from three natural populations, namely Mt. Elgon (28), Gachuthi (27) and Kitui (29). The PCR analysis was performed using Multiplex PCR Mater Mix (QIAGEN) and 10 ng of DNA as described by (Omondi *et al.* 2015). The PCR mix contained a fluorescently labelled M13 primer, M13-tailed forward primer and a reverse primer in the concentration ratio of 0.15:0.01:0.15 µM. For all loci, a touchdown thermal cycling program was used with annealing temperature ranging between 57–55°C. The cycling profile consisted of initial denaturation of 95°C for 15 min followed by 10 cycles at 94°C for 30 s, 57°C for 90 s and 72°C for 60 s (annealing temperature decreasing by 1°C per cycle); and 22 cycles at 94°C for 30 s, 55°C for 90 s and 72°C for 60 s and a single final cycle at 60°C for 30 min using Verity 96 well thermocycler (Applied Biosystems).

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Table 1. Descriptive statistics over all loci for the three natural populations of *Osyris lanceolata* Hochst. & Steud.

DDBJ GenBank accession no.	Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	Na	H_O Mt. Elgon	H_O Gachuthi	H_O Kitui	H_E Mt. Elgon	H_E Gachuthi	H_E Kitui
LC126834	KFOL2	F:AGAATGTCATTTGAAGGCTCGA R:CCTTTCCTCCGTTCTCCTCG	CGTC	178-194	5	0.393	0.556	0.483	0.572	0.626	0.569
LC154965	KFOL7	F: CTGTGCAATGGAGAAGGCCA R:CGCGGGATTGGGATGTCATA	ATT	115-120	2	0.043	0.000	0.000	0.043	0.000	0.000
LC154966	KFOL8	F:GCTGCTTCTACGGTCACTGT R:GTGGTGGATATGGAGGTGGC	CCG	120-130	2	0.000	0.200	0.462	0.073	0.184	0.434
LC126835	KFOL13	F:TCCGAGGAACAGGGACTCTT R:AGCGAAGAACTCATGAGCGAA	AC	139-165	7	0.556	0.148	0.069	0.552	0.139	0.067
LC154967	KFOL15	F:CATTGACGAATTGCATCCCGT R:CGTGAAGTTCAGTGCAAACC	CGC	145-150	2	0.000	0.000	0.000	0.000	0.000	0.000
LC154968	KFOL16	F:TGGAGCCCATTCTCTTTCCTT R:TGCACGTATTCCACATTTCCA	GT	130-160	5	0.107	0.333	0.107	0.103	0.352	0.166
LC126836	KFOL17	F:CATTGACGAATTGCATCCCGT R:CGTGAAGTTCAGTGCAAACC	AG	178-220	21	0.893	0.741	0.793	0.879	0.824	0.863
LC154969	KFOL19	F:GGTAGCGAGCGGTGATATGT R:ACCTAACAACTTGAAGCTCTCCC	TC	200-230	3	0.259	0.000	0.000	0.338	0.000	0.000
LC126838	KFOL24	F:CAACTCGATCGTGCATTGGC R:TCCGCATATCCATTTGGCCG	CT	219-263	15	0.821	0.192	0.276	0.902	0.286	0.452
LC154970	KFOL27	F:CTAAACTGTCAGGGCTTGCT R:ATACCTTAGCTCCCGTTGCG	ATG	225-230	1	0.000	0.000	0.000	0.000	0.000	0.000
LC126839	KFOL28	F:ATAAAGGCCCACGAGCTCAG R:AACATCGCCATGCAGAACAG	CT	245-255	5	0.714	0.000	0.069	0.605	0.000	0.067
LC154961	KFOL29	F:GCTGAATCAGGGACAGGCAT R:GGCCTCGAACAAAGTGCATG	GA	230-250	2	0.000	0.074	0.034	0.000	0.073	0.034
LC126840	KFOL30	F:CTAAACTGTCAGGGCTTGCT R:ATACCTTAGCTCCCGTTGCG	TC	270-306	12	0.643	0.333	0.483	0.614	0.471	0.663
LC126841	KFOL37	F:TTTCTAGAGCTAACATACCTCTGAA R:ATGACCTGGGTGCTTTGCTG	TG	300-340	17	0.889	0.185	0.517	0.853	0.278	0.609
LC126843	KFOL42	F:AGGTCCTCCTGCCTGAGAAT R: CATAGGGCTGTGATGCGTCA	TG	315-337	6	0.308	0.037	0.000	0.277	0.036	0.000
LC126844	KFOL47	F:TTTGATCGTAAATTATAGATGTCCACA R:CCCTTGCTTGATCTCCAGGTA	CA	353-387	15	0.393*	0.731	0.759	0.791	0.771	0.826
LC126845	KFOL48	F:GAGTGCATGGAATTATGTGCGT R:TCGCCATGAGAAGGGTTACT	TC	369-393	12	0.357	0.519	0.621	0.343	0.666	0.519

Note: 5′ M13 tail: TGTAAAACGACGGCCAGT; F, forward sequence; R, reverse sequence; Na, number of observed alleles per locus, H_O heterozygosity observed with *P*-values for the Hardy– Weinberg equilibrium test and significance threshold adjusted using the Bonferroni correction: $*P < 0.05$, H_E heterozygosity expected.

Amplified fragments were analyzed against an internal standard (Liz 600 size standard) on an ABI 3500 (Applied Biosystems). Alleles were visualized and scored using GeneMapper version 5 (Applied Biosystems). The genetic parameters were determined using GenAlex software v 6.4 (Peakall & Smouse 2012). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) was determined using Genepop online software version (http://wbiomed.curtin.edu.au/genepop/).

The number of alleles per locus across the three populations ranged from one (KFOL27) to 21 (KFOL17). Expected heterozygosity ranged from 0.00 (KFOL15, KFOL27 and KFOL29) to 0.902 (KFOL24) in Mt. Elgon population, from 0.00 (KFOL28) to 0.824 (KFOL7, KFOL15, KFOL17, KFOL19 and KFOL27) in Gachuthi population, 0.00 (KFOL7, KFOL15, KFOL19, KFOL27 and KFOL42) to 0.863 (KFOL17) in Kitui population (Table 1). Total paternity exclusion probability (Pe) over all loci was 0.989. Only one pair of loci (KFOL16 - KFOL37) showed significant LD at the 5 % level after Bonferroni correction. Deviation from HWE was detected for one locus (KFOL47) in Mt. Elgon population (Table 1). Out of the 17 markers developed, 12 were polymorphic while five (KFOL7, KFOL8, KFOL15, KFOL27 and KFOL29) were monomorphic.

The 17 microsatellite markers developed are the first reported for *O. lanceolata* and are suitable for population genetic studies due to their high polymorphic characteristics. The markers will be used for studying genetic diversity and population structure across the distribution range, and to assess levels of gene flow

between populations. These studies will be important in designing sustainable management and conservation strategies for the species.

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