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METHODS AND RESOURCES ARTICLE

Development of new SNP and INDEL loci for the valuable African timber species *Lophira alata*

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Abstract

The timber of the species *Lophira alata* (azobe) is very popular for outdoor constructions, which favours its overexploitation and illegal logging. We sampled individuals from Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Gabon, Congo Brazzaville and Republic Democratic of Congo to discover new nuclear and plastidial SNP and INDEL loci through restriction associated DNA sequencing (RADSeq) and low coverage MiSeq genome sequencing. From an initial set of 397 loci, a final set of 126 loci was selected for timber tracking purposes.

Keywords *Lophira alata* · Azobe · Single nucleotide polymorphism · MassARRAY

Lophira alata is a tropical pioneer timber species typical from secondary humid forests. Its distribution ranges from Sierra Leone in Western Africa to Congo in Central Africa. The timber is highly valuable because of its resistance against insects and moulding, which enables it's use in outdoor constructions. Despite a minimum cutting diameter of at least 60 cm in all producer countries, this species is heavily exploited and classified as vulnerable in the IUCN red list.

The genus *Lophira* has been poorly studied until now and only consist of two species: *L. alata* typical from humid forest habitats while the species *L. lanceolata* is mostly found in savannahs. However, when both species are growing in sympatry, species identification is difficult (Biwole et al. 2012). Genetic studies are limited to the development and use of SSRs markers (Ewédjè et al. 2020; Pineiro et al. 2015), which aimed at studying the genetic structure and hybridisation between *L. alata* and *L. lanceolata*. With 10 SSRs, the presence of a cryptic species in *L. alata* was suggested in West Gabon (Ewédjè et al 2020). Thus, little information is available on the diversity and differentiation within both species.

After the entry into force of timber regulations (among others European Timber Regulation and US Lacey Act) requiring that the species identity and the country of origin are declared and correct, tracking tools to control the claims on species and origin received increasing attention (Dormontt et al. 2015). Although many genetic markers have been successfully developed on tropical and temperate species, the low DNA quality obtained from timber strongly hinders the use of SSRs markers in timber tracking (Blanc-Jolivet and Liesebach 2015). By contrast, SNP loci usually provide good results and sets of SNP loci for origin identification have already been developed for other African species (Jardine et al. 2016; Blanc-Jolivet et al. 2017, 2018).

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Cambium or leaf samples from 103 individual trees stemming from West and Central Africa (Table 1) were dried in silicagel in the field. DNA was extracted according to Dumolin et al. (1995). Three and nine samples were selected for respectively, restriction site associated DNA sequencing [RADSeq, (Miller et al. 2007)] and low coverage Illumina MiSeq genome sequencing (Straub et al. 2012). The use of both NGS methods allowed the SNP and INDEL discovery in the nuclear and in the plastidial genomes. A MassARRAY® iPLEX™ design [Assay Design Suite v2.0 (Agena Bioscience™, San Diego, USA)] included loci which showed polymorphism among sequenced individuals and for which the sequences did not show the presence of another SNP locus in a 50 bp neighbourhood. 397 loci were run in 12 multiplexes on a MassARRAY® iPLEX™ platform (Agena Bioscience™, San Diego, USA) using the iPLEX™ GOLD chemistry (Supplementary material S1) on 94 samples (Table 1). Genotyping was done with Typer Viewer v.4.0.24.71 (Agena Bioscience™, San Diego, USA).

In order to select loci showing a differentiation over the species distribution range, we grouped all individuals according to their country of origin. We estimated, when applicable, average diversity, Gregorius' differentiation (Gregorius 1987) and correlation among genetic and geographic distance for each locus (Supplementary material S2). Because most African species show a strong genetic split between Western and Central African populations, we also estimated differentiation parameters within Western African and Central African countries to catch loci showing local genetic differentiation. On the three datasets, we selected the loci with the highest genetic differentiation, the highest genetic-geographic distance correlation, while loci with very low calling rates were excluded (GDA-NT, unpublished). 126 selected loci were included in four MassARRAY®

Table 1 *Lophira alata* samples used for marker development (RADseq, genome skimming) and MassARRAY screening

Country	Region	Latitude	Longitude	MassARRAY	RADseq	MiSeq
Liberia	ICC	5.94558	− 9.13793	6		
Liberia	Nimba	7.491659	− 8.64969	3		
Ivory Coast	Abidjan Park Nat Bancu	5.38578	− 4.04922	5		
Ghana	Benso	5.14737	− 1.87558	7		
Ghana		5.40887	− 2.21982	4		
Nigeria	Aking	5.41616	8.61443	8		
Nigeria	Oban	5.36878	8.43662	4		
Nigeria	Okomu	6.34772	5.34558	6		
Nigeria	Omo, Ogun State	6.833433	4.372517			2
Nigeria	Ore, Ondo State	6.754733	4.721867		1	3
Cameroon	UFA11_05 WIJMA	5.526461	9.011186	2		
Cameroon	Misolé	3.975417	9.920084	1		
Cameroon	Edea	3.830836	10.09161	2		
Cameroon		3.21841	10.25276	1		
Cameroon		3.22071	10.25768		1	1
Cameroon	Pouma	3.853412	10.52089	1		
Cameroon	Matom	3.805921	11.05909	1		
Cameroon	"Arboretum" de Ebogo	3.401505	11.46693	1		
Cameroon	UFA 09-017	2.66518	11.56386	2		
Cameroon	UFA 10031	3.21349	14.27061	8		
Gabon	Carrefour SHM	0.2115	11.93266	7		
Gabon		0.02351	11.01862	2		1
Congo Brazzaville	Jua-Ikié	2.00625	13.9066	3		
Congo Brazzaville	Ngombé	0.69312	15.95535		1	2
Congo Brazzaville	UFA Lopola	3.0594	17.2624	4		
Congo Brazzaville	UFA Loundoungou	2.4237	16.9708	1		
DRC	Winagomo	− 1.9002	17.02326	3		
DRC	Benye	− 1.87318	17.15285	2		
DRC	Province d'Equateur, IFOM	0.48763	18.29331	10		
Total				94	3	9

A total of 103 different individuals were included

iPLEX™ multiplexes (Supplementary material S3), from which 75, 20 and 28 SNP loci were located in the nuclear, chloroplastic and mitochondrial genome respectively. Two chloroplastic and one mitochondrial INDELs were also included. Theoretical performance for the verification of the country of origin reached 86% (self-assignment test with leave-one-Out procedure using the Bayesian criteria of Rannala and Mountain 1997) when we splited the Nigeria and Cameroon individuals according to a STRUCTURE analysis with two putative genetic groups (Pritchard et al. 2000). Indeed, the genetic boarder between West and Central Africa seems to be located between Nigeria and Cameroon where both groups are present in sympatry.

Our new set of 126 loci including both nuclear and plastidial loci will be of great importance for further population genetics and biogeographic studies as well as timber origin tracking in *Lophira alata*.

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