



Genetic diversity of *Dalbergia melanoxylon* Guill. & Perr. populations in the Ferlo zone (Senegal) using nuclear and chloroplast microsatellite markers

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Abstract *Dalbergia melanoxylon* Guill. & Perr. is a highly valued species in the Ferlo region, and its wood is used for various purposes by local populations, including firewood, service wood, and timber. This multifunctionality has weakened its populations, and it has been identified as threatened in previous research. The aim of this study was to investigate the genetic variability of three populations (LC, VC, and YC) along a north–south rainfall gradient, to support the species’ sustainable management and conservation. Eighteen microsatellite markers were used, including eight nuclear (nSSR) and 10 chloroplast (cpSSR) markers, to test their transferability to

D. melanoxylon and study the genetic variability. The results showed that all nSSRs and cpSSRs were transferable, however, only five nSSRs and one cpSSR were polymorphic and used in the downstream analysis. Based on the nSSRs, comparatively high, but non-significantly different, genetic variation was found in all populations (LC: $H_e = 0.650$, VC: $H_e = 0.674$, YC: $H_e = 0.706$), however, indications of inbreeding were detected. For the single polymorphic cpSSR, three haplotypes were identified. Haplotype diversity (H_d) varied in LC ($H_d = 0.536$), VC ($H_d = 0.577$), and YC ($H_d = 0.674$). Low to moderate genetic differentiation was observed between populations ($F_{st\ nSSR} = 0.019$ and $F_{st\ cpSSR} = 0.133$) suggesting recent fragmentation of populations that has not yet impacted genetic variation but could become more pronounced in future generations. This finding underscores the need for further research on effective management and conservation strategies for *D. melanoxylon* populations in the Ferlo region.

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Introduction

The Ferlo sylvo-pastoral zone is one of Senegal’s agro-ecological zones in the northern part of the country (Sy 2009). It has long been characterized by harsh environmental and climatic conditions resulting

from the drought of the 1970s (1970–1989) (Ngom 2013; Sylla et al. 2019). For a long time, this has led to the degradation of natural ecosystems, making timber resources increasingly vulnerable (Bakhoum 2013; Kebe et al. 2020; Dendocker et al. 2020; Sylla 2020; Ndiaye et al. 2022). In the context of climate change and strong demand from local populations for timber and non-timber forest products, some timber species, like *D. melanoxylon* Guill. & Perr. (Ngom 2013; Ndong et al. 2015), are considered as near-threatened in the Ferlo area. This species belongs to the family Papilionaceae and the genus *Dalbergia*. This genus is pantropical and found in over 130 countries, with native distribution in Central and South America, Africa, and Asia (Dasgupta et al. 2023). *Dalbergia* includes species with varied morphologies, ranging from trees and shrubs to woody vines (Vatanparast et al. 2013). It comprises over 250 species (Kiondo et al. 2014; Nakai 2020). However, most of these species are subject to significant anthropogenic pressure and threatened with extinction in their natural habitats due to the high economic value of their quality timber on the international market (Fraveau et al. 2007; Orwa et al. 2009; Ribeiro et al. 2009; Andrianoelina et al. 2009; Li et al. 2021; da Silva Júnior et al. 2022; Xu et al. 2024).

Dalbergia is among the most trafficked wild products globally, with illegal trade estimated between 7 and 23 billion USD annually (Dasgupta et al. 2023). Due to overexploitation, *Dalbergia* was declared globally threatened and was listed in 2017 under Appendix II of the Convention on International Trade in Endangered Species (CITES) to regulate international timber trade (Bhagwat et al. 2015).

Among all species of the genus, only *D. melanoxylon* (African Blackwood or Senegal Ebony) is native to Africa and is found in Senegal, where it was discovered in 1828 by George Samuel Perrottet in the Walo region of Lower Senegal. It has long been valued by Senegalese populations for the traditional manufacture of marabout rosaries (Chevalier 1934). The sylvopastoral zone of Senegal (Ferlo) remains one of the few regions where *D. melanoxylon* still exists as relic populations, although local oral histories emphasize its former abundance before the Sahel droughts of the 1970s.

Dalbergia melanoxylon, a nitrogen fixing timber tree, is an economically and ecologically important tree species for ecosystem functions and local

communities where it provides food, fodder, fuel, pharmacopeia, lumber, timber, and numerous environmental benefits (Kiondo et al. 2014; Ngom et al. 2014; Ndong et al. 2015; Liu et al. 2021). The species produces high quality wood, which is a commodity of international trade for its undeniable ebony-black beauty, hardness and resistance to termite attack (Bryce 1967; Amri et al. 2009; Orwa et al. 2009; Washa 2014; Bell 2023).

Despite its importance at social, economic and ecological levels, the species faces existential crisis due to overexploitation and excessive harvesting and is thus classified as near-threatened (IUCN red list) since 1998 (Nakai et al. 2019; Nakai 2020). Consequently, sustainability of *D. melanoxylon* populations in the current context of climate change, coupled with anthropogenic pressures, is threatened and actions to reverse this tendency of degradation are crucial to ensure the long-term survival and conservation of the species.

For most African tropical species, there is a lack of relevant research and actions detailing effective in situ conservation plans. This is due to limited knowledge of the genetic structure, distribution, population dynamics, and historical characteristics of these species. Regarding *D. melanoxylon*, information on genetic diversity within and between populations is particularly scarce, as well as how this diversity is affected by current habitat changes caused by deforestation and fragmentation. Additionally, few investigations have explored the impact of these changes on population size, inbreeding levels, genetic drift, and loss of genetic diversity (Low et al. 2005). Assessing the diversity and structure of intraspecific genetic variation, as well as acquiring basic knowledge about reproduction and seed dispersal, is crucial for effectively guiding the conservation and management of the remaining populations of this endangered species.

Amri et al. (2009) used RAPD (*Random Amplified Polymorphic DNA*) molecular markers to assess genetic diversity and structure in six natural populations of *D. melanoxylon* in Tanzania. However, the use of these markers has significant limitations due to their low reproducibility and lack of locus specificity (Miah et al. 2013). In contrast, genetic research has been conducted on other species of the *Dalbergia* genus through the development and use of highly informative and reproducible microsatellite markers. These include *Dalbergia monticola* Bosser & R.

Rabev. (Fraveau et al. 2007), *Dalbergia nigra* (Vell.) Allemão ex Benth. (Ribero et al. 2009; da Silva Junior et al. 2022), *Dalbergia hupeana* Hance (Li et al. 2021), *Dalbergia latifolia* Roxb. (Dasgupta et al. 2023) and *Dalbergia odorifera* (Lui et al. 2019; Xu et al. 2024). Consequently, there is a lack of specific genetic markers for *D. melanoxylon*.

In fact, microsatellite markers (Simple Sequence Repeats, SSRs) are one of the most commonly methods used to study genetic variation (Tautz 1989). They have increasingly become the markers of choice for threatened and endangered species because of their co-dominance and highly polymorphic nature, and their cost-effectiveness when processing large numbers of samples (Burger et al. 2018; Li et al. 2021; Moosavi et al. 2022). They are also transferable between related species. This reduces the cost of marker development for certain research (Buzatti et al. 2016). To date, there are no microsatellite markers (nuclear and/or chloroplast) specific to the *D. melanoxylon* species. In contrast, population genetic research of the *Dalbergia* genus using SSR markers has proved effective (Favreau et al. 2007; Andrianolina et al. 2009; Buzatti et al. 2016). In this context, the aim of this study is to use microsatellite markers to investigate genetic variability within and between populations of *D. melanoxylon* along a north–south rainfall gradient at three different sites (Labgar, Velingara Ferlo and Younoufere) in the Ferlo.

Material and methods

Site description and sample collection

Plant material used in this study originated from three sites in the Ferlo, an agro-ecological zone located in the northern part of Senegal, namely: Labgar (LC: 15° 50' N and 14° 48' W; elevation 51 m), Velingara Ferlo (VC: 15° 0' N and 14° 40' W; elevation 25 m) and Younoufere (YC: 15° 16' N and 14° 27' W; elevation 25 m), following a north–south rainfall gradient varying between 200 and 600 mm (Fig. 1). Average annual temperatures in this area vary between 20 and 38 °C and the soils are of the ferruginous type (Ngom 2013; Kebe et al. 2020).

In December 2023, cambium samples were collected from three populations representing the natural distribution area of the species in Senegal (Fig. 1). At

each site, cambium was collected from 30 randomly selected trees and desiccated in silica gel for DNA extraction. Selected trees were spaced at minimum 90–150 m from each other in order to capture as much diversity (allelic richness) as possible and GPS coordinates were recorded for each tree.

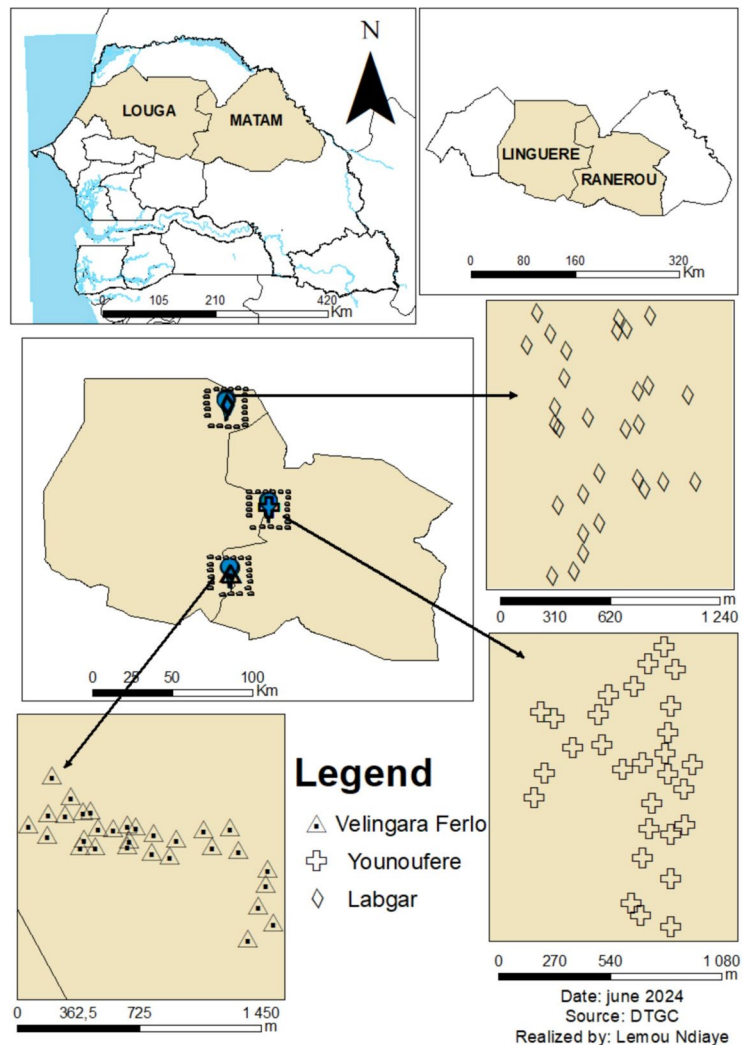
DNA extraction

Genomic DNA was extracted, with 100 mg of dried cambium ground in a bead mill (MM300, Retsch, Haan, Germany). The DNA extraction steps were carried out according to the protocol of the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) for dry matter modified by the addition of 1 ml of polyvinylpyrrolidone (PVP 40 (47%) in a final volume of 100 ml) to the lysis mixture and incubation of the samples at 65 °C overnight. DNA quality and quantity were tested using the Nanodrop 2000/2000c spectrophotometer (Thermo Fisher Scientific) and 1.0% agarose gels stained with Roti@Gelstain (Carl ROTH, Germany), respectively, and visualized under UV light. The DNA solutions were divided into three groups and each group diluted either 1:20, 1:40 or 1:70. The aim was to have diluted samples with a concentration greater than or equal to 5 ng/μl for the PCR amplification process.

Amplification test

Genotyping was performed using a total of 18 microsatellites, including eight nuclear microsatellite markers (nSSRs/MDmCIR) developed by Favreau et al. (2007) for *Dalbergia monticola* Bosser & R. Rabev. and 10 chloroplast markers (cpSSRs/ccmps) described by Weising and Gardner (1999) for dicotyledonous angiosperms (SI Tables 1 and 2). The PCR amplification was done using a PCR mix prepared as described below: 1.0 μl of diluted DNA (with a concentration at least equal to 5 μM/μl), 7.1 μl H₂O, 1.5 μl PCR buffer (10x), 1.5 μl MgCl₂ (25 mM), 1.0 μl dNTPs (2.5 mM each), 1.0 μl M13 primer (5 μM) (6-FAM and HEX), 0.2 μl forward primer (5 μM), 0.5 μl reverse primer (5 μM) and 0.2 μl Taq polymerase (5 units per μl). For cost-efficient PCR with nSSR markers, an M13-specific sequence (5'-CACGAC GTTGTAACGAC-3') was added to the 5' end of each forward primer, so that only the M13 primer had to be labelled with fluorescent dyes (Schuelke 2000).

Fig. 1 Location of the Ferlo area (colored) and three tested populations of *Dalbergia melanoxylon* in Senegal



Also for greater precision a PIG tail sequence (5'-GTTTCTT-3') was added to the 5' end of each reverse primer (Brownstein et al. 1996). PCR was performed in a Biometra thermal cycler (Tprofessional base, Germany).

Nuclear markers

For the nuclear SSRs (MDmCIRA04, MDmCIRA08, MDmCIRA12, MDmCIRB06, MDmCIRC02, MDmCIRC10, MDmCIRD01, MDmCIRD02) we used a program with an initial denaturation temperature of 95 °C for 15 min, followed by 10 cycles of denaturation at 94 °C for 1 min, an annealing temperature of 60 °C for 1 min

(− 1 °C per cycle), extension at 72 °C for 1 min, followed by 25 cycles at 94 °C for 1 min, 50 °C for 1 min and a final extension at 72 °C for 20 min and 16°∞. The nSSR MDmCIRD01 that did not work with this program was subjected to another one with an initial denaturation temperature of 95 °C for 15 min, then 10 cycles of denaturation at 94 °C for 1 min, an annealing temperature of 64 °C for 1 min (− 1 °C per cycle), an extension at 72 °C for 1 min, followed by 25 cycles at 94 °C for 1 min, 54 °C for 1 min and a final extension at 72 °C at 20 min and 16°∞. For the population analysis, only the five markers that showed polymorphism (MDmCIRA08, MDmCIRC02, MDmCIRC10, MDmCIRD01, and MDmCIRD02) were used to analyze the 30 samples from each population.

Chloroplast markers

Amplification of all cpSSRs (*ccmp* 1, *ccmp* 2, *ccmp* 3, *ccmp* 4, *ccmp* 5, *ccmp* 6, *ccmp* 7, *ccmp* 8, *ccmp* 9 and *ccmp* 10) followed a program with initial denaturation at 95 °C for 15 min, then 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, followed by final extension at 72 °C for 20 min and 16°∞. Of these ten, only *ccmp* 7 was polymorphic and used to analyze individuals (30) from each population.

To test the amplification and polymorphism of the markers used, eight samples were randomly selected for PCR. The PCR products obtained were diluted 1:40 for nSSRs and 1:200 for cpSSRs, mixed in equal amounts (HEX and 6-FAM) and separated on the ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, USA) to identify allele sizes. GeneMapper software version 4.1 (Applied Biosystems, Foster City, USA) was then used to evaluate and interpret the microsatellite marker results after PCR, ultimately generating a database for the analysis and interpretation of the results.

Data analysis

Probability of identity for nSSRs was assessed with GenAIEx v.6.5 (Peakall and Smouse 2012). MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to check for the presence of null alleles. The estimates of genetic diversity and differentiation for the co-dominant nSSRs were the number of alleles per locus (N_a), the effective number of alleles (N_{ae}), the allelic richness (A_R) on samples standardized to 58 gene copies, the observed (H_o) and expected (H_e) heterozygosity, the inbreeding coefficient (F_{is}) and the fixation index (F_{st}). All these parameters were calculated for all loci combined and for each locus separately using SPAGeDi 1.5d software (Hardy and Vekemans 2002). To estimate potential significant differences in genetic variation between populations, the Kruskal Wallis test (Kruskal and Wallis 1952; R Core Team 2023) was conducted using R version 4.3.3.

To confirm the absence of family structures (groups of closely related trees) within each population, fine-scale genetic structure was assessed using the software SPAGeDi 1.5d (Hardy and Vekemans 2002). The Loiselle kinship coefficient (F , Loiselle

et al. 1995) was estimated for each pair of samples, and the slope b_F was obtained by regressing the average kinship coefficients per distance class against the logarithm of spatial distances. The strength of genetic structure was then calculated as $Sp = b_F / (F_1 - 1)$, where F_1 is the average kinship coefficient of pairs of individuals in the first distance class (SI Fig. 4). The significance of the results was tested using 10,000 random permutations of individuals among locations.

Genetic differentiation was also obtained with SPAGeDi using an AMOVA approach following Weir and Cockerham (1984) and probability values were obtained by permutation of individuals among populations (number of permutations 10,000). Haplotype analysis version 1.05 (Eliades and Eliades 2009) was used to calculate haplotype frequency (Supplementary file Table 3) and estimate genetic diversity parameters such as number of haplotypes detected (A), number of private haplotypes (P), effective number of haplotypes (N_e), haplotype richness (R_h), genetic diversity (H_d), mean genetic distance between individuals (D^2_{sh}) and genetic differentiation (F_{st}) (Table 3).

The genetic structure of the populations was assessed and quantified in two steps. Principal coordinate analysis (PCoA) was performed in GenAIEx v.6.5 (Peakall and Smouse 2012) based on Nei's unbiased genetic distances (Nei 1978) between individual samples. Also, STRUCTURE v.2.3.4 (Pritchard et al. 2000) based on the admixture model with correlated allele frequencies was used with a running-in period of 10,000 iterations, followed by 10,000 iterations of the Markov chain with five repetitions for $K=1-5$ (Supplementary file Fig. 1). In order to deduce the most likely number of clusters (K), Evanno's method (Evanno et al. 2005) implemented in STRUCTURE SELECTOR was used (Li and Liu 2018) (SI Figs. 2 and 3). STRUCTURE results were processed using the CLUMPAK pipeline (Kopelman et al. 2015), and cluster assignment graphs were generated by averaging the results across all replicates for each value of K .

Results

All 18 microsatellite markers (eight nSSRs and ten cpSSRs) used in this study were transferable to *D. melanoxylon* (Table 1). Out of the eight nSSR

Table 1 Transferability of nuclear and chloroplast microsatellite markers to *Dalbergia melanoxylon*

Name of Locus	Repeat motif	Sequence (5'–3')	Fluorescent Dye	SSR type	Allele size range <i>D. melanoxylon</i> (bp)	Ta (°C)
MDmCIRA08 ¹	(CT) ₂₁	F: M13-TCCATTCTCCTTCCACAG R: PIG-TCCTCCACTATTATTGCCC	HEX	nSSR	120–195	60
MDmCIRC02 ¹	(GT) ₁₈	F: M13-CTCTCTTCTCCAATCCAC R: PIG-CTACCAGTCACTCACCTTC	HEX	nSSR	259–262	60
MDmCIRC10 ¹	(CT) ₂₇	F: M13-AGGGGGAATACAGAAAGGC R: PIG-AGAGATGGGTGGGTCAAAG	HEX	nSSR	202–276	60
MDmCIRD01 ¹	(TC) ₁₇	F: M13-GCACAAGATATGGAGATGGAG R: PIG-AATGAAAAGGGTGAGCAGC	6-FAM	nSSR	183–263	64
MDmCIRD02 ¹	(CT) ₁₉	F: M13-CGCACTACTCCCTTCCTTC R: PIG-CGACCACACTCTCCAACAC	6-FAM	nSSR	132–144	60
<i>ccmp</i> 7 ²	(A) ₁₃	F: CAACATATACCACTGTCAAG R: ACATCATTATTGTATACTCTTTC	HEX	cpSSR	148–150	50

¹Fraveau et al. (2007), ²Weising and Gardner (1999), *M13* 5'-CACGACGTTGTAAAACGAC-3', *PIG* 5'-GTTTCTT-3', *T_a* annealing temperature, *SSR* microsatellite marker, *nSSR* nuclear microsatellite marker, *cpSSR* chloroplast microsatellite marker

markers tested on *D. melanoxylon* individuals, five were polymorphic, while only one of the ten cpSSRs tested was polymorphic (SI Tables 1 and 2). Probability of identity for the five nSSRs was 0.0001. The results from MICROCHECKER indicate that MDmCIRA08 is the only locus with a high probability of containing null alleles. For the other loci, although some methods (notably Brookfield 2) suggest a degree of uncertainty, the majority of indices indicate the absence of null alleles. Due to the presence of null alleles, MDmCIRA08 was excluded from the calculation of F_{is} and F_{st} (SI Table 3).

The only polymorphic cpSSR (*ccmp* 7) revealed an allele size range of 148–150 bp in *D. melanoxylon*.

Genetic variation in nSSRs

Sampling of groups of closely related trees was avoided as confirmed by the analysis of fine-scale genetic structure (SI Fig. 4). The average number of alleles (N_a) for all loci ranged from 6.6 for the VC population to 8.0 for the LC population, while average allelic richness (A_R) varied from 6.560 (VC) to 7.970 (LC). For individual loci, N_a and A_R ranged from 4 to 13 (Table 2). Average values of inbreeding coefficients were positive and significantly different from zero for all populations across all markers ($F_{isLC}=0.117$, $p=0.030$; $F_{isVC}=0.144$, $p=0.009$; $F_{isYC}=0.107$, $p=0.022$). The individual markers showed a wide range of positive F_{is} values

(0.011–0.504) with the exception of MDmCIRD02 and MDmCIRC10 markers showing a negative F_{is} value for LC and YC populations ($F_{is}=-0.022$ and -0.172 , respectively).

For each individual marker, the expected heterozygosity (H_e) varied from 0.359 to 0.892 (mean $H_e=0.650$) for LC, from 0.458 to 0.844 (mean $H_e=0.674$) for VC and from 0.400 to 0.860 (mean $H_e=0.706$) for YC. The observed heterozygosity (H_o) ranged from 0.367 to 0.828 (mean $H_o=0.563$) for LC, from 0.333 to 0.800 (mean $H_o=0.593$) for VC and from 0.200 to 0.867 (mean $H_o=0.605$) for YC (Table 2). Genetic variation parameters were not significantly different among populations. According to the Kruskal Wallis test, high probabilities ($P>0.05$) were found after testing all parameters.

The fixation index (F_{st}) for all loci shows a very low but significant mean value ($F_{st}=0.019$, $p=0.003$). The pairwise test for F_{st} between populations showed significant differences between LC and VC ($F_{st}=0.017$, $p=0.026$) and between LC and YC ($F_{st}=0.034$, $p=0.001$). However, there was no significant difference between VC and YC ($F_{st}=0.006$, $p=0.186$).

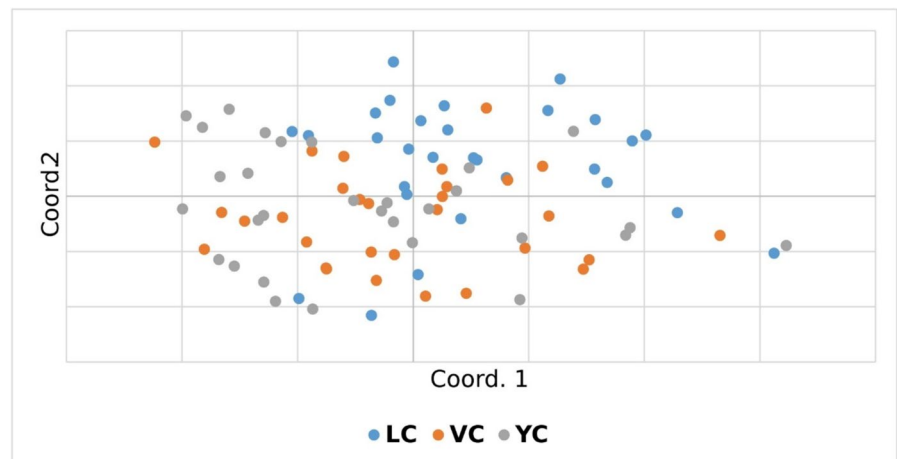
The pattern observed in the Principal coordinate analysis (PCoA), with nSSRs based on Nei's unbiased genetic distance, showed no clear grouping of individuals. However, a slight distinction was noted for the LC population compared with VC and YC (Fig. 2). The STRUCTURE analysis also failed

Table 2 Parameters of genetic variation within each population of *Dalbergia melanoxylon* according to polymorphic markers

Locus	LC (N=30; P=200 – 300 mm; A=51 m)															
	F_{st}	P_{Fst}	N	N _a	N _{ae}	A _R	H _e	H _o	F_{is}	P_{Fis}						
MDmCIRA08	–	–	30	8	2.72	7.93	0.632	0.500	–	–						
MDmCIRC02	0.032	0.043	30	6	3.04	5.97	0.672	0.433	0.359	0.001*						
MDmCIRC10	–0.000	0.414	30	6	3.29	6.00	0.697	0.690	0.011	0.905						
MDmCIRD01	0.014	0.050	30	13	9.19	13.00	0.892	0.828	0.073	0.270						
MDmCIRD02	0.042	0.006*	30	7	1.56	6.96	0.359	0.367	–0.022	0.899						
Averages across loci	0.019	0.003*	30	8	3.96	7.97	0.650	0.563	0.117	0.030*						
Locus	VC (N=30; P=300–500 mm; A=25 m)								YC (N=30; P=300–600 mm; A=25 m)							
	N	N _a	N _{ae}	A _R	H _e	H _o	F_{is}	P_{Fis}	N	N _a	N _{ae}	A _R	H _e	H _o	F_{is}	P_{Fis}
MDmCIRA08	30	9	6.39	8.97	0.844	0.800	–	–	30	9	5.57	8.90	0.821	0.600	–	–
MDmCIRC02	30	4	1.84	4.00	0.458	0.367	0.203	0.141	30	4	1.67	4.00	0.400	0.200	0.504	0.000*
MDmCIRC10	30	7	3.89	6.90	0.743	0.733	0.014	0.864	30	9	3.87	8.90	0.742	0.867	–0.172	0.056
MDmCIRD01	30	9	6.28	8.93	0.841	0.733	0.130	0.114	30	8	7.13	8.00	0.860	0.759	0.120	0.138
MDmCIRD02	30	4	1.93	4.00	0.483	0.333	0.314	0.018*	30	7	3.43	6.97	0.708	0.600	0.155	0.106
Averages across loci	30	6.6	4.07	6.56	0.674	0.593	0.144	0.009*	30	7.4	4.33	7.35	0.706	0.605	0.107	0.022*

LC Labgar (population 1), VC Velingara ferlo (population 2), YC Younoufere (population 3), P annual precipitation, A altitude, N number of samples, N_a number of alleles per locus, N_e effective number of alleles per locus, A_R alleles richness, H_o observed heterozygosity, H_e expected heterozygosity, F_{is} inbreeding coefficient, P_{Fis} probability of significance of inbreeding between individuals, * significantly different from zero, F_{st} fixation index and P_{Fst} = significance of genetic variation between population

Fig. 2 PCoA based on Nei's unbiased genetic distance between all *Dalbergia melanoxylon* samples from three populations



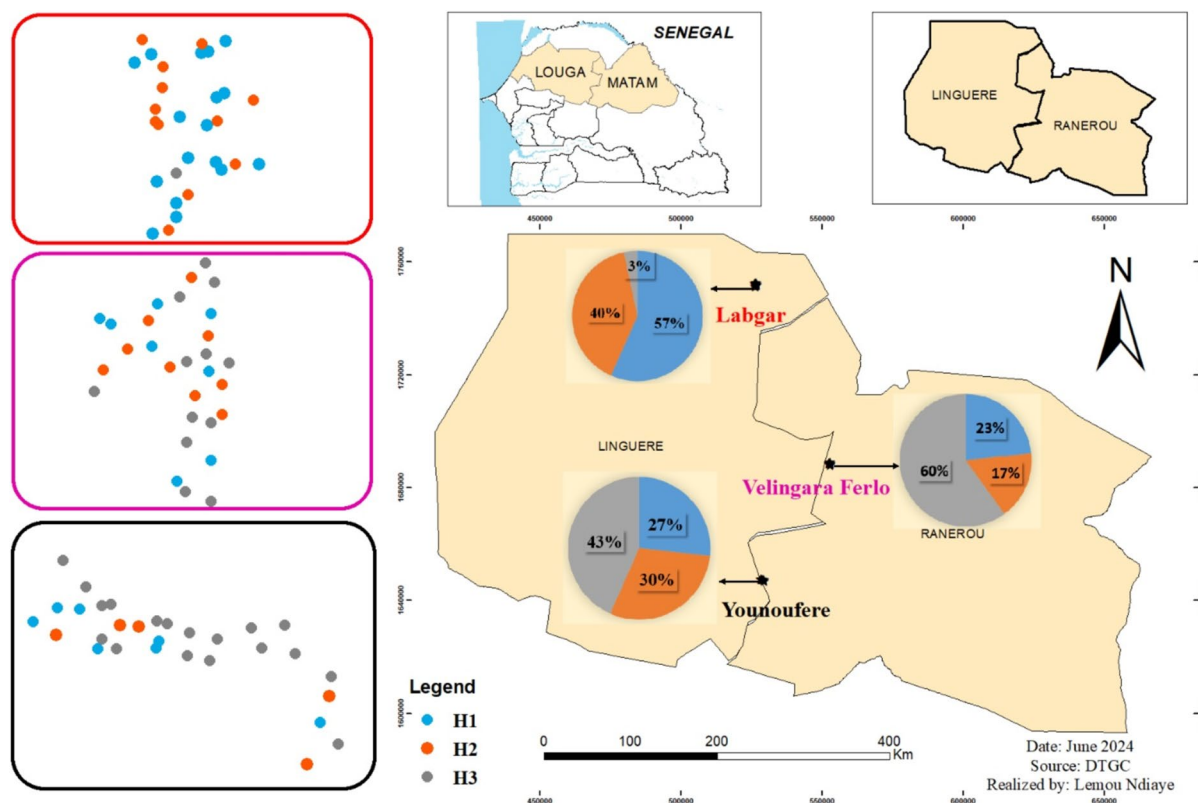


Fig. 3 Representation of haplotype frequencies in the three populations of *Dalbergia melanoxylon*

Table 3 Haplotype diversity parameters by population

Population	N	A	P	N_e	R_h	H_d	D^2_{sh}	F_{st}
LC	30	3	0	2.074	2	0.536	0.653	0.194
VC	30	3	0	2.261	2	0.577	1.446	0.137
YC	30	3	0	2.866	2	0.674	1.391	0.073
Mean	30	3	0	2.400	2	0.595	1.163	0.133

N sample size in each population, A number of haplotypes detected in each population, P number of private haplotypes, N_e effective number of haplotypes, R_h haplotypic richness, H_d Haplotype diversity, D^2_{sh} mean genetic distance between individuals, F_{st} genetic differentiation among populations

to identify genetic clusters or genetic structure (SI Fig. 1). The highest probability of our data was observed for $K=3$ (SI Fig. 2 and Fig. 3).

Genetic variation at cpSSRs

The calculation of haplotype diversity showed that there is no difference in haplotype richness and no private haplotypes for the three populations (Table 3). Each population is composed of the three haplotypes

detected in the 90 study samples (Table 3). The level of haplotype diversity (H_d) detected in all three populations is 0.595 on average, but the analysis in each population shows values between 0.536 for LC to 0.674 for YC. Estimation of the genetic distance between individuals (D^2_{sh}) in the three populations shows an average value of 1.163. However, individuals within the LC population were genetically more similar ($D^2_{sh}=0.653$) compared with those belonging to VC ($D^2_{sh}=1.446$) and YC ($D^2_{sh}=1.391$)

(Table 3). In addition, moderate genetic differentiation was observed between populations ($F_{st}=0.133$).

Analysis of haplotype frequencies within the studied populations shows a clear variation which differentiates LC from the other populations (Fig. 3 and SI Table 4). The most striking differences are those of haplotype H1 (148 bp), which is present in 57% of samples in the LC population and only 23% and 27%, respectively, in the VC and YC populations. Haplotype H3 (150 bp) with a very low frequency in LC (3%), is also very frequent in VC (60%) and YC (43%).

Overall, the results show that for both types of microsatellite markers, the three populations of *D. melanoxylo*n exhibit a high level of genetic variation within each population, accompanied by a low level of genetic differentiation between them.

Moreover, the lack of clear clustering in the PCoA and STRUCTURE analyses indicates genetic homogeneity between the LC, VC, and YC populations, likely due to significant gene flow. However, the limited number of microsatellite markers used in this study may partially account for the absence of genetic structuring. Therefore, these results should be interpreted with caution, and further analyses with a larger set of markers are needed to confirm these findings.

Positive F_{is} values in each population suggest the presence of inbreeding among individuals, despite the outcrossing and self-incompatible nature of the species. These signatures of inbreeding might be explained by reproductive dynamics or environmental factors limiting pollen flow.

Discussion

The present study is the first to present estimates of genetic diversity in *D. melanoxylo*n based on molecular markers in Senegal. The results showed that all 18 microsatellite markers (eight nSSRs and ten cpSSRs) used in this study were transferable to *D. melanoxylo*n. Among these, 60% of nSSRs and 10% of cpSSRs were polymorphic. This observed low level of polymorphism in cpSSRs is due to their generally more conserved nature compared to nSSRs. In other words, cpSSRs are less likely to vary rapidly because the chloroplast genome is often uniparentally inherited and subjected to more uniform selective pressure (Weising and Gardner 1999). Conversely, nSSRs are

naturally highly variable, inherited from both parents with recombination, which increases genetic diversity. The transfer of polymorphic nSSR markers is therefore more likely between closely related species such as *D. monticola* (Madagascar) and *D. melanoxylo*n (Senegal), two species of the same genus originating from the African continent (Andrianoelina et al. 2009; Li et al. 2021). Buzatti et al. (2016) have also shown a high transferability and polymorphism of these same nSSRs (87.5%) from *D. monticola* to *Dalbergia miscolobium* Benth. from Brazil.

Estimation of genetic variation using putatively neutral nSSRs showed that populations of *D. melanoxylo*n from North to South Ferlo have comparatively high levels of genetic variation, while the level of genetic variation is not significantly different for the three populations (LC (north): $N_a=8$; $A_R=7.970$; $H_o=0.563$ and $H_e=0.650$, YC (center): $N_a=7.4$; $A_R=7.350$; $H_o=0.605$ and $H_e=0.706$) and VC (south): $N_a=6$; $A_R=6.560$; $H_o=0.593$ and $H_e=0.674$). The values of genetic diversity parameters, are almost identical for the three populations (LC, YC, and VC). Additionally, there is no significant genetic differentiation between them regardless of their geographical location. This genetic similarity suggests that LC, YC and VC populations were likely connected in the past by gene flow through pollen and seed dispersal, allowing a constant exchange of genes. As a result, the populations have very similar genetic backgrounds.

In contrast, higher genetic diversity using the same markers, was reported for *D. monticola* in Madagascar, depending on the sampling area (Andrianoelina et al. 2009): North ($N_a=8.200$, $A_R=6.430$, $H_o=0.630$ and $H_e=0.760$); North Central ($N_a=12$, $A_R=6.780$, $H_o=0.640$, $H_e=0.810$); Central ($N_a=16.200$, $A_R=6.610$, $H_o=0.600$, $H_e=0.790$); and South ($N_a=19.200$, $A_R=7.280$, $H_o=0.700$ and $H_e=0.790$).

In addition, the low level of genetic differentiation observed at nSSRs ($F_{st}=0.019$) confirmed by the pattern observed in the PCoA analysis, indicating no genetic and geographic structure in our populations, and the comparatively high genetic variation within populations at nSSRs suggests efficient gene exchange in the past. The moderate signs of inbreeding coefficient (F_{is}) obtained within each population, points to a decrease of genetic variation and adaptive potential if no further measures are taken.

Forest fragmentation appears to be recent based on genetic data and has not yet had a large impact on genetic variation within populations. However, signs of inbreeding are indicative of negative impacts in future generations, manifesting as increased inbreeding levels and reduced genetic variation in future generations of trees. These results corroborate those of Andrianoelina et al. (2009), who stated that positive F_{is} values were more frequent in fragmented than in non-fragmented populations of *D. monticola*, due to reduced population size in these forests and isolation of adult individuals.

The Ferlo region, where the studied populations originate from, has experienced significant ecosystem degradation following a major drought in the 1970s (Ngom 2013; Sylla et al. 2019). This environmental disruption, combined with current climatic instability and increasing human pressure on woody resources, including *D. melanoxylon*, has led to a reduction in forest cover (Bakhoun 2013; Kebe et al. 2020; Dendocker et al. 2020; Sylla 2020; Ndiaye et al. 2022). As a result, *D. melanoxylon* is now considered as threatened species, with populations reduced to small isolated groups, thereby decreasing its genetic diversity (Ngom 2013; Ndong et al. 2015). In the face of climate change, the sustainable management and protection of natural resources, particularly of timber species like *D. melanoxylon*, is a critical challenge in the Ferlo. The region's low erratic and unpredictable rainfall, coupled with ongoing human exploitation, further threaten the species' long-term viability.

Our results suggest that historically, populations of *D. melanoxylon* in the Ferlo were interconnected through gene flow, enabling the dispersal of seeds and pollen before the drought of the 1970s. However, this drought caused severe environmental changes, which led to regression of natural populations of *D. melanoxylon* making local populations and natural resources highly vulnerable. This could explain, the current genetic structure and signatures of inbreeding observed in our study. Although there is not yet significant genetic differentiation between populations, signs of habitat fragmentation are apparent, reflecting the long-term impacts of environmental and anthropogenic stresses on *D. melanoxylon*.

In the context of management and conservation of the species, this homogeneity could facilitate the development of conservation strategies, but it also

underscores the importance of maintaining this connectivity to prevent a potential reduction in genetic diversity in the future. Continuous monitoring of changes in genetic structure will be crucial to adapting conservation strategies in response to future ecological threats.

Conclusion

This study stressed the importance of using DNA markers in assessing the genetic diversity and structure of the threatened *D. melanoxylon* tree species to support conservation efforts. We reported comparatively high levels of genetic diversity within tested populations and low genetic differentiation among them. Our results suggest recent habitat fragmentation. Signatures of inbreeding in each population suggest a decrease of genetic variation and adaptive potential in future tree generations. This information is useful for designing an effective forest conservation program for this threatened species. In fact, given the fact that natural regeneration is generally low in this region due to adverse environmental conditions (low rainfall, overgrazing, livestock damage...) coupled with small population size which will ultimately lead to inbreeding with its negative effects on the vigour and adaptability of *D. melanoxylon* populations, we recommend the establishment of seed orchard/clonal seed orchards (ex situ conservation) in addition to the in situ conservation by the designation of protected areas.

Thus, future research on optimizing seed germination, cutting/grafting techniques as well as on physiological responses of *D. melanoxylon* under drought conditions is needed to support conservation and domestication of the species for future uses and generations.

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Author contribution LM, AMD, OG contributed to the study conception and design. LM and GV performed material preparation in the lab and data collection. LM, AMD, GV,

MM, and OG carried out the statistical analyses. LM, AMD, GV, MM, and OG analyzed and interpreted the data, and contributed to writing of the paper. All authors (LM, AMD, OG, MM, GV, DN and TM) have read and approved the final manuscript.

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Data availability Microsatellite data will be archived in in the Goettingen Research Online repository (<https://doi.org/https://doi.org/10.25625/MGKPZA>).

Declarations

Conflict of interest The authors declare no competing interests.

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