

Evaluation of Genetic Diversity in *Dalbergia Melanoxylon* Populations Using Random Amplified Polymorphic DNA Markers.

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Abstract: African Blackwood *Dalbergia melanoxylon* (Guill. & Perr.) is a tropical tree with high-quality wood and one of the most expensive timbers in the world. Randomly amplified polymorphic DNA (RAPD) analysis was used to determine the genetic diversity within and among six natural populations of *D. melanoxylon* in Tanzania. A total of 60 individual plants were analysed using 14 most informative RAPD primers which amplified 87 scored bands showing 73.6 % polymorphism. Cluster analysis using unweighted pair group method with arithmetic average (UPGMA) formed four major clusters which illustrated that most individuals from a given population tended to cluster together and were therefore more genetically similar than individuals from different populations. Most of the genetic variations were found to be high within population 63.6% and low among populations 36.4%. The genetic diversity information of *D. melanoxylon* populations reported in this study is very important component for efficient conservation and effective management of its genetic resources.

Key words: *Dalbergia melanoxylon*, Conservation, genetic diversity, RAPD markers

INTRODUCTION

Dalbergia melanoxylon (African Blackwood) belongs to the family Leguminosae and is one of the most important indigenous trees in Tanzania. The species has a high-quality wood, which is used for the manufacturing of musical instruments and decorative objects such as carvings that are popular to foreign visitors^[20]. As a result of the valuable products derived from *D. melanoxylon*, high exploitation pressure has been exerted to the extent of threatening its genetic diversity and future existence in its natural habitats^[13,20]. *D. melanoxylon* is considered by the IUCN Red List as Lower Risk/near threatened (LR/nt) species^[41]. Moreover, this species has serious reproductive limitations, both asexually and sexually with very low germination capacity^[28,30]. Therefore information on genetic variability is important for successful management and conservation of the species.

Genetic diversity in tropical tree species is being lost rapidly due to the process of deforestation, which has reduced the size of natural populations and eliminated local populations^[31]. The situation is particularly grave with regards to some species of economic importance, such as the *D. melanoxylon*. Knowledge about genetic diversity and population genetic structure offers baseline information for formulating effective conservation plans, and can often

provide novel, conservation-relevant insights^[26,31]. Preservation of genetic variation and evolutionary processes in viable populations has an ecological importance of preventing potential extinction for the species^[36].

Different methodologies using molecular markers are widely used to analyse the pattern of variations within and among natural populations of tree species. Among the various markers used, random amplified polymorphic DNA (RAPD) is the most popular DNA based markers^[6,22,2,19]. These markers are the technically least demanding and offer a fast method for providing information from a large number of loci, particularly in species where no studies have previously been undertaken. Moreover, in terms of results, the diversity assessed with RAPDs is comparable with that obtained with restriction fragment length polymorphism (RFLP) or allozymes^[9].

Previous studies in assessing diversity of *D. melanoxylon* have used morphological methods^[25]. However, the use of morphological markers for estimating genetic diversity in plants have limitations due to effect of stage of development or environment compared to molecular markers^[29]. Little information is available on genetic diversity of *D. melanoxylon* using molecular markers. Genetic diversity of *D. melanoxylon* in Ubena and Mkundi populations in eastern part of Tanzania has previously been assessed

using Random Amplified Microsatellite (RAMS) molecular markers ^[13]. However, their study was characterised by low level of sampling intensity, few populations and very small number of primers that might have failed to capture all genetic variations in *D. melanoxyton*. Such limitations calls for further studies of assessing genetic diversity in *D. melanoxyton* using RAPD markers with increased sampling intensity and increased number of primers. RAPD markers provide a larger number of polymorphism bands that are useful for the analysis of genetic diversity, often using fast, simple and reliable protocols that minimize the amount and quality of DNA required ^[6].

RAPD markers have been successfully used to determine genetic diversity of various trees including *Melia volkensii*^[36], *Vitellaria paradoxa*^[3], *Brachylaena huillensis*^[24], *Oroxylum indicum*^[18]. This work was carried out using RAPD markers to evaluate the genetic diversity within and among six populations of *D. melanoxyton* in order to suggest appropriate conservation and management strategies.

MATERIALS AND METHODS

Study Populations and Sampling Methodology: Leaf samples were collected from six natural

similarity matrix of individuals was calculated using Dice coefficient [81] and cluster analysis run on Sequential, Agglomerative, Hierarchical and Nested (SAHN) using the Unweighted Pair Group Method with Arithmetic average (UPGMA). Principal component analysis (PCA) was performed using EIGEN procedures and the 2-D scatter plot generated. Genetic diversity was estimated using Shannon's information index (H_j) (Lewontin, 1972) using POPGENE software Version 1.32 [42]. The level of genetic diversity detected by different primers in each population was calculated as (H_j); the average diversity of populations for each primer (H_{pop}); and total diversity in the whole sample considered together (H_{sp}). It was then possible to calculate the proportion of diversity within populations (H_{pop}/H_{sp}) and among populations $G_{ST} = [(H_{sp} - H_{pop})/(H_{sp})]$. The percentages of polymorphic loci (%P) were also calculated in each population.

RESULTS AND DISCUSSION

Fourteen most informative RAPD primers out of 30 primers screened were selected for DNA amplification reactions because they yielded many highly repeatable polymorphic bands. These primers generated a total of 87 reproducible RAPD bands with fragments ranging from 200 to 1200 bp, of which 64 (73.6%) were polymorphic and 23 (24.4%) were monomorphic. The bands per primer produced ranged from 4 to 9, and the average band loci per primer was 6.2 (Table 2). Primers, KFP04 and KFP23 gave the highest degree of polymorphic bands 83.3% and 85.7% respectively. Primers KFP08 and KFP17 gave low polymorphism indices (60%).

Cluster Analysis: The UPGMA dendrogram based on Dice's similarity coefficient obtained using the 87 RAPD markers exhibited four main clusters, cluster I – IV, when truncated at (0.80 coefficient) 80% similarity level (Fig 1). Cluster I was the largest cluster which consisted three sub-clusters namely Ia, Ib and Ic. Sub cluster Ia consisted of 80% (8/10) of individuals from population MDL, Sub cluster Ib with 90% (9/10) of individuals from population MBZ and sub cluster Ic with 90% (9/10) of individuals from population KBH. Cluster II was formed by all individuals from population UBN in which individuals were at 84% similarity level. In cluster III, 90% (9/10) of individuals were from population MND at 82.4% similarity level, while one individual (59MND) from this population was outside the principal cluster. Cluster IV was formed by all individuals from Mikumi (MKM) population at about 84.8% similarity level.

The dendrogram (Fig 1) illustrated the genetic structure of these populations, showing that most

individuals from a given population tend to cluster together and are therefore more genetically similar than individuals from different populations. Based on the UPGMA dendrogram, similarity coefficients ranged from 72% to 97.6 % among the 60 samples of *D. melanoxylon*. The most similar individuals were individuals 44 and 49 for population MND, with 97.6% similarity. Individuals (MND51) and (MND53) for population MND were the least similar individuals, with 86.67% similarity (Fig 1).

Principal Component Analysis (PCA): Principal Component Analysis (PCA) was performed to discriminate all individuals of the six different populations (Fig 2). The PCA revealed also four major clusters, and confirmed the results of the cluster analysis. The first two principal components (Eigenvectors) explained 82.4% of the total variation (1st 78.3% and 2nd 4.1%).

Genetic Diversity Estimates of the Populations: Genetic diversity measures of each population (H_j) for each primer and mean values with respect to all primers were calculated (Table 3). The RAPD primers varied in their power to detect genetic diversity of populations. The highest genetic diversity values were obtained with primer KFP23, in populations UBN, and KBH, whilst the lowest were obtained with primers KFP05 and KFP24. However, the latter did not detect diversity in population MKM. Ranges of mean values for Shannon's information showed that the highest and the lowest genetic diversity values were obtained in the populations MND (0.323) and MKM (0.173), respectively (Table 3).

The mean diversity within the six populations of *D. melanoxylon* was $H_{pop} = 0.244$, and the total diversity of the whole sample population was $H_{sp} = 0.388$ (Table 3). The proportion of average diversity within populations (H_{pop}/H_{sp}) was 63.6%, and ranged between 34.1% (primer KFP24) and 91.10% (primer KFP04). The proportion of diversity among populations was 36.4%. All primers detected more variability within than among populations.

Considering each population separately, percentage of polymorphic loci revealed that the highest number of polymorphic loci was 47 (54%) found in population MND, while the lowest number of polymorphic loci was 32 (36.8%) found in population MKM (Table 4). A total of 73.6% polymorphic loci were found in the six populations indicating that *D. melanoxylon* had high genetic diversity.

Discussion: Analyses of the RAPD markers using cluster analysis, Principal Component Analysis (PCA) and Shannon's diversity measure techniques

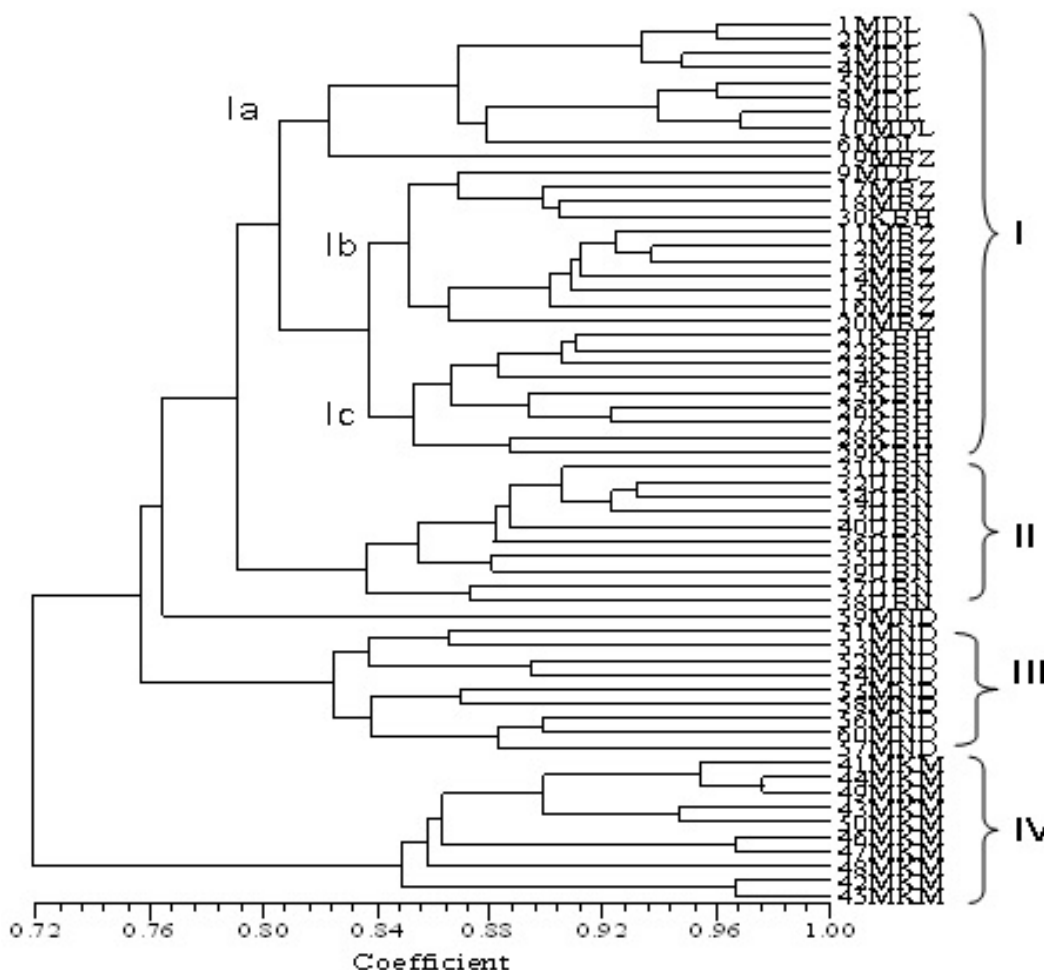


Fig. 1: UPGMA dendrogram computed from genetic similarity matrix using Dice's similarity coefficient of 60 individuals sampled across six populations of *Dalbergia melanoxylon*. Populations are denoted as: MDL=Madale, MBZ=Mbezi, KBH=Kibaha, UBN=Ubena, MKM=Mikumi and MND=Mkundi. Numbers I - IV are clusters.

demonstrated similar interpretations of the genetic structure and diversity of *D. melanoxylon* populations. The study revealed a high level of polymorphism (73.6%) in the six populations of *D. melanoxylon*. Considering each population separately, this value ranged from 36.8% to 54%. The percentage of polymorphic bands within populations of *D. melanoxylon* is comparatively similar to other species, such as *Allium aeseae* (40–63%;^[37] and *Erodium paularense* (44–51%;^[26].

The results of the partition of variation within populations and among populations of *D. melanoxylon* by Shannon's information measure showed high genetic diversity within populations and low genetic diversity among populations. It has been reported that a close association exists between breeding system and the distribution of genetic diversity among population^[14,22,32]. Inbreeding species are generally characterized

by high levels of genetic differentiation among populations, whilst outcrossing species tend to retain considerably low variability among populations^[15]. According to Frankham *et al.*^[11], reproductive system is the most important factor in determining the genetic structure of plant populations along with the effects selection, genetic drift, mutation, and migration. Studies of the biology of flowering and pollination in *D. melanoxylon* indicate that it is an outcrossing^[1,28]. Hamrick *et al.*^[16] and Nybom and Bartish^[33] reported that long-lived woody plants, outcrossing in natural ranges are likely to have high genetic variability within and low among populations. All of which are characteristics typical of *D. melanoxylon*.

Genetic differentiation estimates among populations for outcrossing species have been found to range between 15% - 38%, when RAPD markers were analysed using Shannon's index^[4]. The genetic

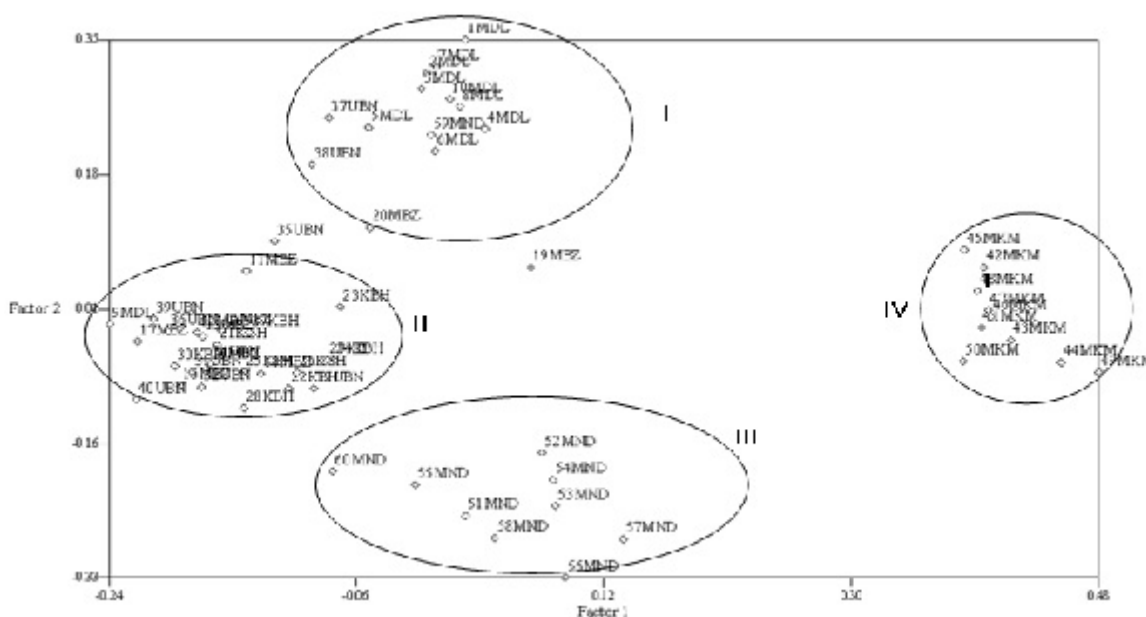


Fig. 2: Principal Components Analysis (2-D) using RAPD data of 60 individuals of *Dalbergia melanoxylon*. Populations are denoted as: MDL=Madale, MBZ=Mbezi, KBH= Kibaha, UBN=Ubena, MKM=Mikumi and MND=Mkundi. Numbers I - IV are clusters.

diversity among populations of *D. melanoxylon* of 36.4% is towards the higher limit and is in agreement with the outcrossing nature of the species. There are other outcrossing plant species that present similar values of diversity among populations, e.g. *Cedrela odorata* (37%;^[12], *Alkanna orientalis* (32%;^[40], *Silene tatarica* (36.9%;^[38], and slightly higher values, as in the other endangered plant species, *Caesalpinia echinata* (41%;^[5]. The genetic diversity within populations of *D. melanoxylon* of 63.6% obtained with RAPD markers is also within the range that has been estimated for other tree species reported by Newton *et al.*^[31] and Bouvet *et al.*^[3] in which Shannon's diversity index within populations ranged from 42% to 89.7%. An explanation for the rather high, within-population diversity for these species is the outcrossing nature of species which is the most important determinant of population genetic structure^[15].

The UPGMA dendrogram illustrated that most individuals from a given population tend to cluster together and are therefore more genetically similar than individuals from different populations. Populations of Madale, Mbezi and Kibaha grouped together in one cluster, which may be due to effective gene flow among the three populations. This is because these populations from coastal areas are not separated by large geographical distance from each other approximately 25 km apart. Populations of Ubena, Mkundi and Mikumi are from inland areas and relatively separated by large geographical distance from each other approximately 100 km apart. The movement

of genes among populations has a significant influence on the distribution of genetic variation^[15]. The geographical separation might have contributed to limited gene flow among different studied populations of *D. melanoxylon*. This pattern of isolation by distance is often observed in insect-pollinated tree species when long distances within the natural range are considered^[2,21].

High genetic diversity was revealed by RAPD markers within Ubena and Mkundi populations. The results are in agreement with findings of Hamisy and Hantula^[13] using Random Amplified Microsatellite (RAMS) markers, who found high genetic diversity within these populations. Genetic diversity for Mikumi National Park population was low (0.173) as well as low in percentage of polymorphism loci (36.8%) compared to other populations (Table 3 and Table 4). This low polymorphism within Mikumi National Park population could be due to the restricted gene flow and small population size. Distribution range and population size have been identified as major correlates of within-population genetic variation in some tropical tree species, with restricted populations showing significantly less variation than those with broader distribution^[23]. Mikumi National Park is a protected area; therefore confinement of *D. melanoxylon* population in the national park may also have compromised diversity due to decreased potential for long distance seed dispersal through anthropogenic activities. The natural stands of *D. melanoxylon* in Mikumi national park continue to decline; only a few

Table 1 Description of sampling sites of *D. melanoxylon* populations

Provenance/ population	Code	Latitude	Longitude	Altitude (m.a.s.l.)
Madale	MDL	S 06°41' 43.5"	E 039°08' 41.6"	104
Mbezi	MBZ	S 06°47' 28.1"	E 039°07' 16.6"	137
Kibaha	KBH	S 06°46' 17"	E 038° 57' 13.0"	142
Ubena	UBN	S 06°36' 03.6"	E 038° 09' 24.0"	350
Mkundi	MND	S 06°40' 03.6"	E 037° 39' 24.0"	475
Mikumi	MKM	S 07° 06' 16.1"	E 037°14' 10.5"	522

Table 2 Number of scored bands per primer and polymorphism in *D. melanoxylon*

Primer Code	Sequence of primer 5' to 3'	Total number scored bands	Number of polymorphic bands	Polymorphism (%)
KFP01	GGC TCG TAC C	9	7	77.7
KFP02	CGT CCG TCA G	4	3	75.0
KFP03	GTT AGC GGC G	6	4	66.7
KFP04	CGG AGA GTA C	6	5	83.3
KFP05	CCT GGC GAG C	9	6	66.7
KFP06	TCC CGA CCT C	4	3	75.0
KFP07	CCA GGC GCA A	5	4	80.0
KFP08	ACG CGC TGG T	5	3	60.0
KFP10	ACG GTG CGC C	9	7	77.7
KFP16	GCA TGG AGC T	4	3	75.0
KFP17	CCG AAG CCC T	5	3	60.0
KFP18	ACC CAT TGC G	8	6	75.0
KFP23	GCT CGT CAA C	7	6	85.7
KFP24	ACT CGT AGC C	6	4	66.7
Total		87	64	73.6

Table 3: Estimates of the genetic diversity parameters (Shannon's information index) for the six populations of *Dalbergia melanoxylon* for 14 RAPD primers.

Primer code	Genetic diversity (H_j) of each population						H_{pop}	H_p	H_{pop}/H_p	$G_{ST} = H_p - H_{pop}/H_p$
	MDL	MBZ	KBH	UBN	MKM	MND				
KFP01	0.145	0.091	0.205	0.149	0.129	0.888	0.268	0.449	0.597	0.403
KFP02	0.222	0.156	0.156	0.328	0.328	0.391	0.264	0.437	0.603	0.397
KFP03	0.242	0.115	0.104	0.115	0.104	0.261	0.157	0.426	0.367	0.633
KFP04	0.291	0.365	0.507	0.384	0.384	0.287	0.368	0.404	0.911	0.089
KFP05	0.056	0.201	0.089	0.211	0.083	0.229	0.144	0.217	0.663	0.337
KFP06	0.312	0.245	0.297	0.320	0.151	0.323	0.275	0.341	0.808	0.192
KFP07	0.232	0.125	0.245	0.138	0.132	0.136	0.168	0.330	0.509	0.491
KFP08	0.254	0.178	0.125	0.269	0.125	0.259	0.202	0.488	0.413	0.587
KFP10	0.219	0.184	0.216	0.239	0.179	0.345	0.231	0.458	0.503	0.497

Table 3: Continue

KFP16	0.328	0.267	0.284	0.256	0.051	0.172	0.226	0.390	0.580	0.420
KFP17	0.161	0.281	0.294	0.379	0.205	0.333	0.276	0.315	0.876	0.124
KFP18	0.218	0.315	0.374	0.261	0.232	0.306	0.284	0.341	0.833	0.167
KFP23	0.308	0.495	0.511	0.523	0.322	0.386	0.424	0.469	0.904	0.096
KFP24	0.048	0.127	0.178	0.192	0	0.224	0.128	0.376	0.341	0.659
Mean	0.217	0.225	0.256	0.269	0.173	0.323	0.244	0.388	0.636	0.364

Populations are denoted as: MDL=Madale, MBZ= Mbezi, KBH= Kibaha, UBN=Ubena, MKM= Mikumi, MND=Mkundi; H_{pop} average diversity within populations; H_{sp} total diversity; H_{pop}/H_{sp} proportion of diversity within populations; $G_{ST} = (H_{sp} - H_{pop})/H_{sp}$ proportion of diversity among populations.

Table 4: Percentage of polymorphic loci in populations of *Dalbergia melanoxylon*

	Population						
	MDL	MBZ	KBH	UBN	MKM	MND	All populations
Number of Polymorphism	37	41	39	41	32	47	64
Polymorphism							
loci (%)	42.5	47.1	44.8	47.1	36.8	54.0	73.6

have been reported still existing in the national park [17]. Genetic diversity of tree species has also been found in many studies to vary directly with population size [39].

Plant species, especially the perennials like trees, rely on the available genetic diversity for stability and survival under the ever-changing environments. Populations with high level of genetic variation are valuable since they offer a diverse gene pool from which gene conservation and improvement programs can be made [24]. The decrease in the genetic diversity limits a species ability to keep pace with the changing selection pressure and their roles in the ecological and evolutionary development of the biosphere. [35,24].

Consequently maintenance of genetic diversity is important as the diversity carries forward both ecological adaptation and microevolution. The over exploitation of *D. melanoxylon* thus threatens their genetic diversity and hence might limit their ecological and evolutionary development of the remaining populations. Therefore, knowledge of the levels and distribution of genetic diversity is important for designing conservation strategies for threatened and endangered species [10]. Based on the information available for *D. melanoxylon* the following conservation strategies are proposed.

In situ conservation plan that defines areas free from significant disruption for the genetically most diverse populations of *D. melanoxylon* in Mkundi, Ubena, Kibaha, Madale and Mbezi. This would guarantee that gene flow is occurring to maintain most of the species' genetic variation. Since the present study has shown that Mikumi national park population is less diverse, efforts should be made for *ex situ* conservation based on seeds and stem cuttings collections from the population and subsequent reintroduce them into their original parental localities.

Domestication of *D. melanoxylon* in field plots also proposed as means of conservation and utilisation that will ensure local people needs for cash incomes from sale of tree products. Domestication will decrease pressure on the few natural populations and protect its genetic diversity.

In conclusion, results from this study indicate that RAPDs are sufficiently informative and powerful to assess genetic variability in *D. melanoxylon*. Estimates of genetic variation reported herein provide a basis for the *in situ* conservation and exploitation of genetic resources in this species. With knowledge of the available genetic structure, an appropriate strategy for sampling and propagation of *D. melanoxylon* may be easily formulated for *ex situ* conservation of the genetic resources of the species.

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