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# 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

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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. melanoxylon*. Such limitations calls for further studies of assessing genetic diversity in *D. melanoxylon* 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. melanoxylon* 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 [8] 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 (*I*) (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

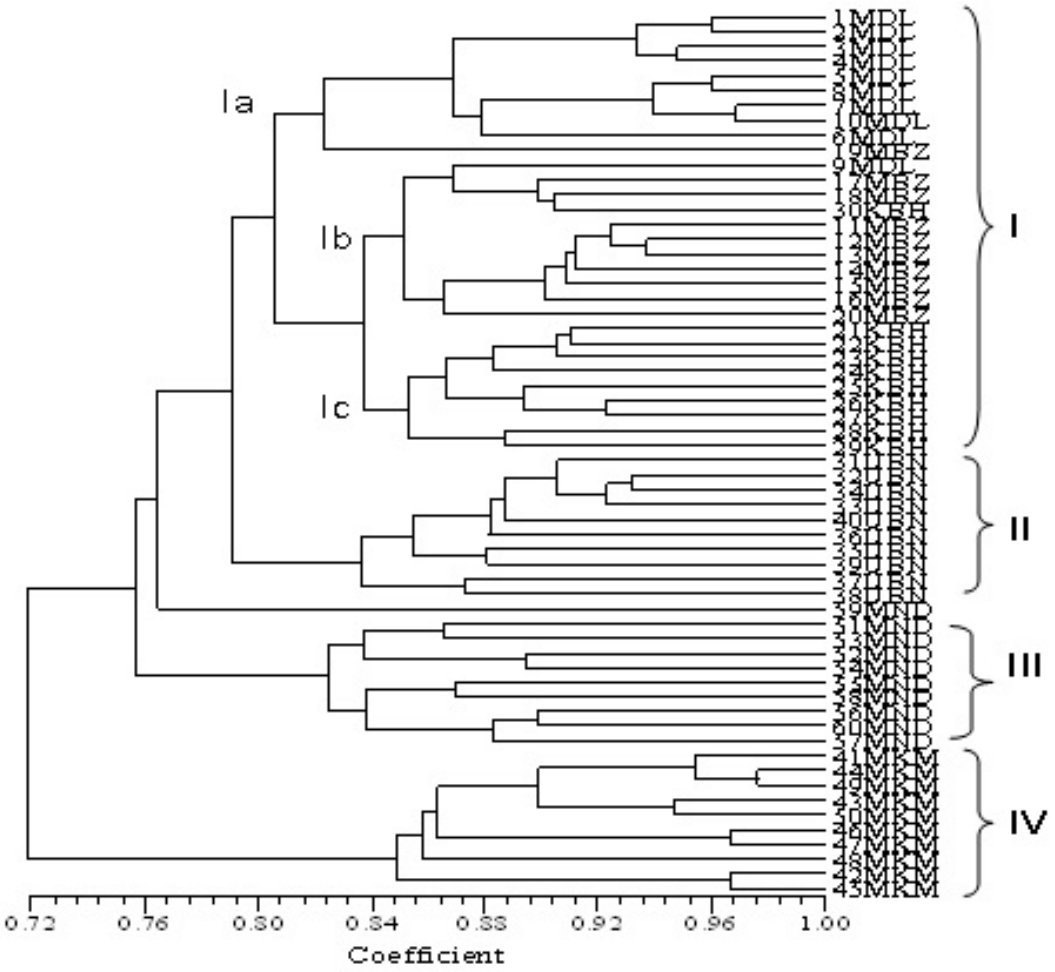


**Fig. 1:** UPGMA dendrogram computed from genetic similarity matrix using Dice's similarity coefficient of 60 individuals sampled across six populations of Dalbergia melanoxylon. Populations are denoted as: MDL=Madale, MBZ=Mbezi, KBH=Kibaha, UBN=Ubena, MKM=Mikumi and MND=Mkundi. Numbers I - IV are clusters.

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 aaseae* (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

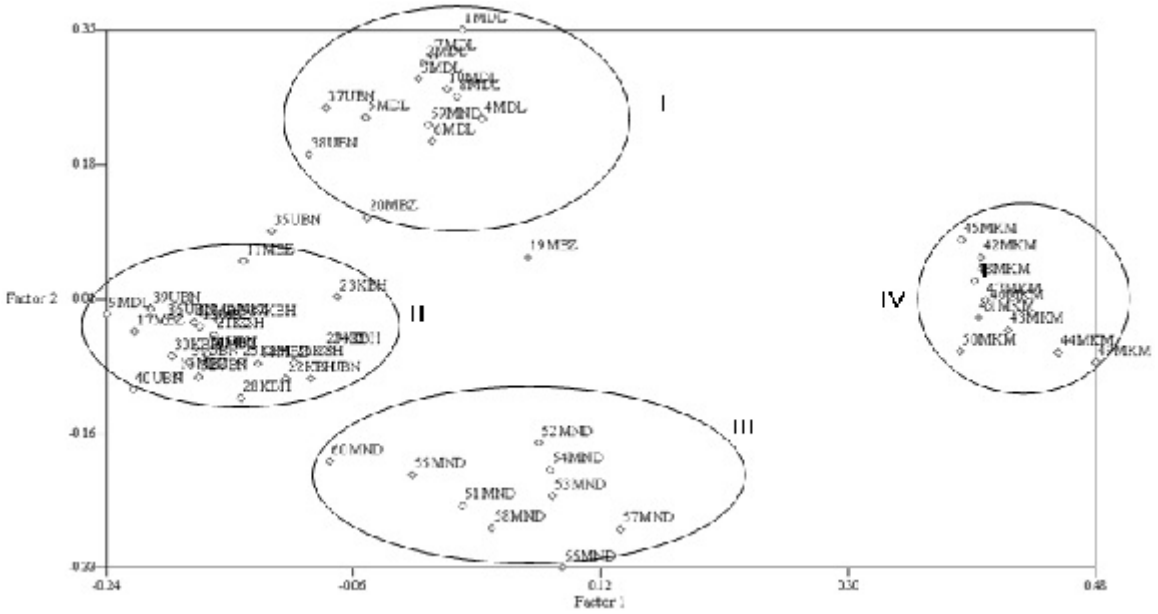


**Fig. 2:** Principal Components Analysis (2-D) using RAPD data of 60 individuals of Dalbergia melanoxylon. Populations are denoted as: MDL=Madale, MBZ=Mbezi, KBH= Kibaha, UBN=Ubena, MKM=Mikumi and MND=Mkundi. Numbers I - IV are clusters.

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$^0$4I' 43.5", | E 039$^0$08' 41.6" | 1O4 |
| Mbezi | MBZ | S 06$^0$47' 28.1" | E 039$^0$07'16.6" | 137 |
| Kibaha | KBH | S 06 $^0$46' 17" | E 038$^0$ 57' 13.0" | 142 |
| Ubena | UBN | S 06 $^0$36' 03.6" | E 038$^0$ 09' 24.0" | 350 |
| Mkundi | MND | S 06 $^0$40' 03.6" | E 037$^0$ 39' 24.0" | 475 |
| Mikumi | MKM | S 07$^0$ 06' 16.1" | E 037$^0$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 |
| KFPO2 | 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 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MDL | MBZ | KBH | UBN | MKM | MND | $H_{pop}$ | $H_{sp}$ | $H_{pop}/H_{sp}$ | $G_{ST} = H_{sp}$-$H_{pop}/H_{sp}$ |
| KFP01 | 0.145 | 0.091 | 0.205 | 0.149 | 0.129 | 0.888 | 0.268 | 0.449 | 0.597 | 0.403 |
| KFPO2 | 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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## REFERENCES

1. Albrecht, J., 1993. Tree Seed Hand book of Kenya. Forestry Seed Centre Muguga, Kenya Germany Development Cooperation Kenya Forestry research institute/GTZ, Nairobi, pp: 34- 54.
2. Bekessy, S.A., T.R. Allnutt, A.C. Premoli, A. Lara, R.A. Ennos, M.A. Burgman, M. Cortes and A.C. Newton, 2002. Genetic variation in the vulnerable and endemic Monkey Puzzle tree, detected using RAPDs. Heredity., 88: 243–249.

3. Bouvet, J.M., C. Fontaine, H. Sanou and C. Cardi, 2004. An analysis of the pattern of genetic variation in *Vitellaria paradoxa* using RAPD markers. Agroforest. Syst., 60: 61–69.
4. Bussell J.D., 1999 The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea* (Lobeliaceae). Mol. Ecol., 8: 775-789.
5. Cardoso, M.A., J. Provan, W. Powell, P.C.G. Ferreiras and D.E. de Oliveira, 1998. High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinioideae). Mol. Ecol. 7: 601–608.
6. Chunyang, L., 2000. RAPD analysis of genetic variation in *Eucalyptus microtheca* F. Muell. populations. Hereditas, 132: 151-156.
7. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant mini preparation: version II. Plant Mol. Biol. Reporter, 1(14): 19-21.
8. Dice, L.R., 1945. Measures of the amount of ecologic association between species. Ecology, 26: 297-302.
9. Esselman, E.J., D.J. Crawford, S. Brauner, T.F. Stuessy, G.J. Anderson and O.M. Silva, 2000. RAPD marker diversity within and divergence among species of Dendroseris (Asteraceae: Lactuceae). Am. J. Bot., 87(4): 591–596.
10. Francisco-Ortega, J., A. Santos-Guerra, S.C. Kim and D.J. Crawford, 2000. Plant genetic diversity in the Canary Islands: A conservation perpective. Am. J. Bot., 87: 909-909.
11. Frankham, R., J.D. Ballou and D.A. Briscoe, 2004. Introduction to Conservation Genetics. Cambridge University Press, New York, USA, pp: 87-98.
12. Gillies, A.C.M., J.P. Cornelius, A.C. Newton, C. Navarro, M. Hernández and J. Wilson, 1997. Genetic variation in Costa Rican populations of the tropical timber species *Cedrela odorata* L., assessed using RAPDs. Mol. Ecol., 6: 1133–1145.
13. Hamisy, W.C. and J. Hantula, 2002. Characterization of genetic variation in African Blackwood, *Dalbergia melanoxylon* using Random Amplified Microsatellite (RAMS) method. Plant Genetic Resources and Biotechnology in Tanzania. Part 1: Biotechnology and social aspects. Proceedings of the Second National Workshop on Plant Genetic Resources and Biotechnology, 6-10 Arusha, Tanzania., pp: 108 – 117.
14. Hamrick, J.L. and M.J.W. Godt, 1996. Effects of the life history traits on genetic diversity in plant species. Phil. Trans. R. Soc.,. B. 351: 1291–1298.
15. Hamrick, J.L. and M.J.W. Godt, 1990. Allozyme diversity in plant species. In: Plant population genetics, breeding and genetic resources (Brown AHD, Clegg MT, Kahler AL and Weir BS, eds.). Sinauer Associates, Sunderland, pp: 145-162.
16. Hamrick, J.L., M.J.W. Godt, D.A. Murawski and M.D. Loveless, 1991. Correlations between species traits and allozymes diversity: implications for conservation biology. In: Falk D, Holsinger K (eds). Genetics and Conservation of Rare Plants. Oxford University Press: New York, pp: 75–86.
17. Hawkins, D.M., H.V.M. Lyaruu and G.W. Norton, 1996. *Dalbergia melanoxylon* in Mikumi National Park. In: Proceedings of The Maputo Workshop: Towards Conservation and Responsible Use of *Dalbergia melanoxylon*. 6-9 November 1995, Flora & Fauna International., pp: 44-51.
18. Jayaram, K. and M.N.V. Prasad, 2008. Genetic diversity in *Oroxylum indicum* (L.) Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker. Afr. J. Biotechnol., 7(3): 254-262.
19. Jena, S., P. Sahoo, S. Mohanty and A.B. Das, 2004. Identification of RAPD markers, *in situ* DNA content and structural chromosomal diversity in some legumes of the mangrove flora of Orissa. Genetica., 122: 217-226.
20. Jenkins, M., S. Oldfield and T. Aylett, 2002. International Trade in African Blackwood. Fauna & Flora International, Cambridge, UK.
21. Juchum, F.S., J.B. Leal, L.M. Santos, M.P. Almeida, D. Ahnertand and R. X. Corrêa, 2007. Evaluation of genetic diversity in a natural rosewood population (Dalbergia nigra Vell. Allemão ex Benth.) using RAPD markers. Gen. Mol. Res., 6(3): 543-553.
22. Lacerda, D.R., M.D. Acedo, J.P. Filho and M.B. Lovato, 2001. Genetic diversity and structure of natural populations of *Plathymenia reticulata* (Mimosoideae), a tropical tree from the Brazilian Cerrado. Mol. Ecol., 10: 1143-1152.
23. Loveless, M.D., 1992. Isoenzyme variation in tropical trees: Patterns of genetic organization. New. Forest., 6: 67-94.
24. Machua, J., G. Muturi and J. Gicheru, 2007. Genetic diversity within *Brachylaena Huillensis* (O. Hoffim.) populations in Kenya: implications for conservation of a wood Carving tree. J. Discov. Innov., 19(1): 32-36.
25. Malimbwi, R.E., E.J. Luoga, O. Hofstad, A.G. Mugasha and J.S. Valen, 2000. Prevalence and standing volume of *Dalbergia melanoxylon* in coastal and inland sites of Southern Tanzania. J. Tropic. For. Sci., 12: 336-347.
26. Martin, J.P., J.E. Hernandez Bermejo, 2000. Genetic variation in the endemic and endangered *Rosmarinus tomentosus* Huber-Morath & Maire (Labiatae) using RAPD markers. Heredity., 85: 434–443.
27. Martín, C., M.E. González-Benito J.M. and Iriondo, 1997. Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. and Izco. Mol. Ecol., 6: 813–820.

28. Mbuya, L.P., H.P. Msanga, C.K. Rufo, A. Birnie and B. Tengnas, 1994. Useful trees and shrubs of Tanzania. Identification, propagation and Management of Agricultural and Pastoral community. Regional soil conservation unit, Swedish international development authority, Nairobi, pp: 542.
29. Moyib, O.K, M.A. Gbadegesin, O.O. Aina and O. A. Odunola, 2008. Genetic variation within a collection of Nigerian accessions of African yam bean (*Sphenostylis stenocarp*) as revealed by RAPD primers. Afr. J. Biotechnol., 7(12): 1839-1846.
30. Msanga, H.P., 1998. Seed germination of indigenous trees in Tanzania. Including notes on seed processing, storage and plant uses. Natural Resources Canada, Canadian Forest Service, Northern Forest Centre, Edmonton.., pp: 292.
31. Newton, A.C., T.R. Allnutt, W.S. Dvorak, R.F. Del Castillo and R.A. Ennos, 2002. Patterns of genetic variation in *Pinus chiapensis,* a threatened Mexican pine, detected by RAPD and mitochondrial DNA RFLP markers. Heredity., 89: 191–198.
32. Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol. Ecol., 13: 1143–1155.
33. Nybom, H. and I.V. Bartish, 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant Ecol. Evol. Syst., 3: 93–114.
34. Rohlf, F.J., 2000. NTSYS-pc *Numerical taxonomy and multivariate analysis system Version 2.1*. Setauket, New York: Exeter Software.
35. Runo, M.S., G.M. Muluvi and D.W. Odee, 2004. Analysis of genetic structure in *Melia volkensii* (Gurke.) populations using random amplified polymorphic DNA Afr. J. Biotechnol., 3(8): 421-425.
36. Segarra-Moragues, J.G., M. Palop-Esteban, F. González-Candelas and P. Catalán, 2005. On the verge of extinction: genetics of the critically endangered Iberian plant species, *Borderea chouardii* (Dioscoreaceae) and implications for conservation management. Mol. Ecol., 14: 969-982.
37. Smith, J.F. and T.V. Pham, 1996. Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). Am. J. Bot., 83: 717–726.
38. Tero, N., J. Aspi Siikamaki, P.A. Jakalaniemi, and J. Tuomi, 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. Mol. Ecol., 12: 2073-2085.
39. Travis, S.E., J. Maschinski, and P. Keim, 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *Cremnphylax*, a critically endangered plant, using AFLP markers. Mol. Ecol., 5: 735–745.
40. Wolff, K., S. El-Akkad and R.J. Abbott, 1997. Population substructure in *Alkanna orientalis* (Boraginaceae) in the Sinai Desert, in relation to its pollinator behaviour. Mol. Ecol., 6: 365–372
41. World Conservation Monitoring Centre, 1998. *Dalbergia melanoxylon*. In: IUCN 2006. 2006 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 08 November 2006.
42. Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao, 1999. Popgene Version 1.31. University of Alberta, Edmonton, Alberta, Canada.