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# RAPD Variation Within and Among Natural Populations of African Cherry (*Prunus Africana*) From Ethiopia

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**Abstract:** In order to facilitate reasoned scientific decisions on its management and conservation, genetic analysis of six populations of *P. africana* sampled from six different geographical regions of Ethiopia was performed using Random Amplified Polymorphic DNA (RAPD) markers. Seventy six percent of the loci studied revealed polymorphism for the whole data set. Within population diversity estimated by using Nei's gene diversity estimates ranged from 0.307 (Chilimo) to 0.150 (Bulki), with a mean of 0.234. Genetic differentiation between populations was estimated with Nei's  $G_{ST}$  (0.264) and analysis of molecular variance (AMOVA) based  $F_{ST}$  (0.257), which appears to be slightly higher than the average values obtained from various RAPD based studies on outcrossing and long-lived species. Genetic relationships among the populations were examined. The resulting tree separated the six populations into two primary clusters which somewhat reflects their geographical locations. Data suggested that conservation approach of *P. africana* should consider each population separately.

**Keywords:** Conservation, Ethiopia, Polymorphism, Population, *Prunus africana*, RAPD

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## 1. Introduction

*Prunus africana* (Hook. F.) Kalkaman (Rosaceae) (syn. *Pygeum africanum*), is a montane forest tree species naturally occurs in most of tropical African countries; from Ethiopia in the North, to South Africa in the south, as far West as Nigeria, and on outlying islands as Madagascar [1]. It is the only species in the genus *Prunus* that is native to Africa [2].

*P. africana* is commercially important for its bark, which is in high demand in the treatment of benign prostatic hyperplasia, and for its wood as a good source of timber [1]. This has led to serious destruction of the species throughout Africa, leading to concerns on the long-term sustainability of harvesting and the conservation of this species. As a result, *P. africana* is listed as endangered under appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; [3]). In addition, the FAO Panel of Experts on Forest Gene Resources lists *P. africana* as one of 18 top priority species for action in Africa [4].

In Ethiopia, despite the species is not yet exploited for its

bark, the natural populations of this species are seriously threatened by excessive logging and illegal tree felling activities that has led to a widespread deforestation [5]. *P. africana* is now found as a remnant of isolated and scattered populations [6], which are still under severe pressure of human impacts. Clearly, for preserving the remaining natural populations of this valuable species, urgent conservation efforts are needed.

At the forefront of management decisions concerning endangered species is the necessity to understand the biology of the species and the factors acting against its natural survival. Population genetics provides critical guidance for interpreting the present status and future prognosis of threatened species as it allows to study relevant parameters such as genetic diversity within populations, the distribution of this genetic variability in the species that occurred in fragmented populations and, to infer historical process that have molded the current species structure. Population genetics analysis of endangered species has been identified as one of the main priorities for

conservation biology [7] and the integration of these data with those from other disciplines will allow one to make sound decisions for the appropriate conservation of endangered species [8].

In this paper, the patterns of random amplified polymorphic DNA (RAPD: [9]) variation in six natural populations of Ethiopian *P. africana* were analyzed to pinpoint its genetic status in order to facilitate reasoned scientific decisions on its management and conservation.

RAPD was used for this study because the technique has previously been used successfully in discerning genetic variation within and among populations of a diverse array of tree species [e.g. 10, 11, 12], and that RAPDs requires no prior sequence information on the target genome. The objectives were to quantify levels of genetic diversity within populations and genetic differentiation among populations of *P. africana* in Ethiopia and to identify genetically distinct units for conservation purpose.

## 2. Materials and Methods

### 2.1. Plant Material

Six populations of *P. africana*, with eight individuals in each population, sampled from six different localities of Ethiopia were used in this study. Selections of the populations were made to represent geographic regions where natural populations of *P. africana* are found within the country. Table 1 shows the geographical information of each sampled population.

In each population, fresh leaf samples were collected from young, actively growing branches of eight individual standing trees and placed in tubes containing silica gel to dry and preserve samples. To decrease the probability of collecting samples from individuals of the same clone, sample trees within each population were chosen with 100m distance apart from each other. Figure 1 shows the geographical regions in Ethiopia from where samples were taken.

Table 1. List of *P. africana* populations with their geographical information.

Locality	Altitude(m.a.s.l.)	Latitude, Longitude	Status of stand
Tepi	1600	6°59'N, 35°14'E	Protected natural Forest
Bedele	2005	8°27'N, 36°20'E	Remnant trees in patchy, coffee planted forest and farm lands
Lepisi (Arsi)	2300	7°20'N, 38°45'E	Few trees in patchy natural forests and on agricultural lands
Chilimo	2200	9°04'N, 38°09'E	Protected natural Forest
Bulki	2420	6°20'N, 36°50'E	Remenant trees in farm lands and home yards
Agere Mariam	2000	5°36'N, 38°20'E	Few trees in patchy natural forests, and in agricultural and grazing lands.

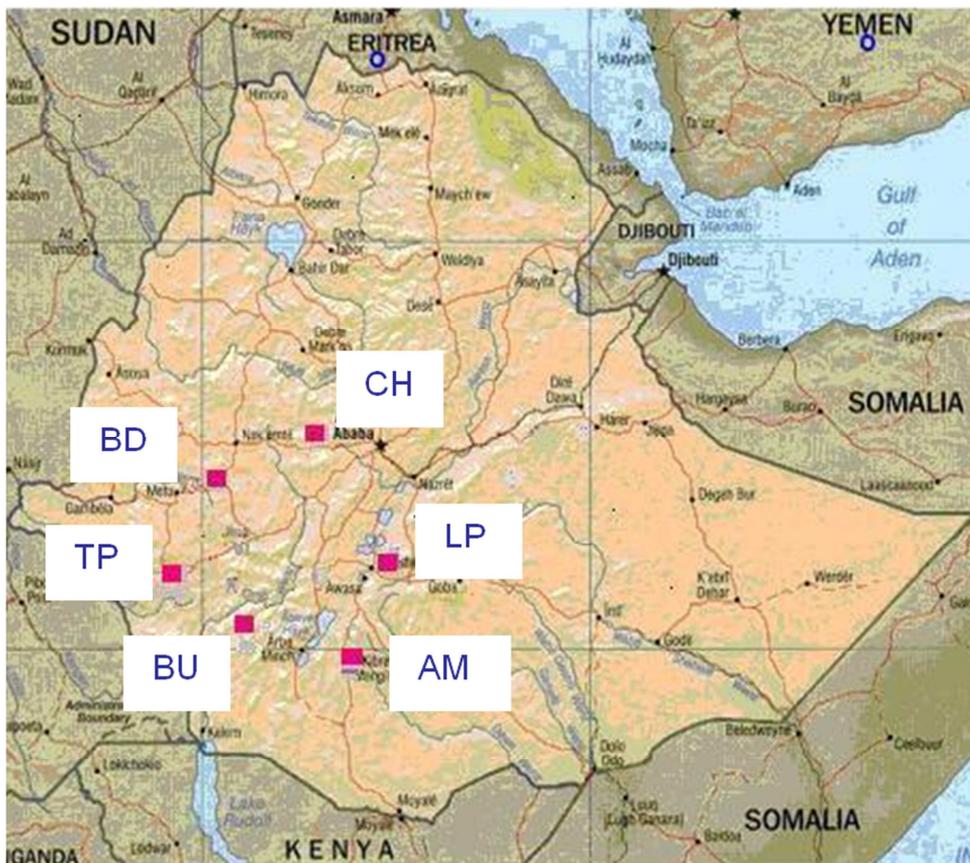


Figure 1. Map of Ethiopia showing the 6 regions from where the *Prunus africana* populations were sampled (Shaded red) (AM= Agere Mariam; BD=Bedele; BU= Bulki; CH=Chilimo; LP= Lepisi and TP= Tepi).

## 2.2. DNA Extraction

Silica-gel dried young leaves of *P. africana* were used for total genomic DNA extraction. DNA was extracted by using a modified version of CTAB method as described by [13].

## 2.3. RAPD PCR Amplification and Electrophoresis

### 2.3.1. Primer Screening and RAPD PCR Amplification

A number of protocols that have been used for plant DNA amplification were tested in a T3 PCR machine (Biometra, Personal) and the one with best amplification profiles was chosen. After choosing the PCR protocol, 7 RAPD primers (series OPB (OPB15, OPB 14, OPB, 11, OPB 8) and OPC (OPC16, OPC 10 AND OPC 4), Operon Technologies, Inc.) were evaluated using DNA of two randomly chosen samples with the objective of screening primers that can detect polymorphism, show clearly resolvable banding patterns and amplify larger number of loci per sample. Accordingly 4 primers that fulfill the above criteria were screened.

For the RAPD reaction, 2.5 µl sample DNA were used as a template in a total volume of 20µl containing 1X reaction buffer (50mM KCl, 10mM Tris-HCl, PH= 8.4, 2.5mM MgCl<sub>2</sub>, 0.1µ g/µl of gelatin), 3.5 mM MgCl<sub>2</sub>, 10 ng/ µl primer, 0.1mM of each dNTPs, 0.6 units of *Taq* DNA polymerase from Peq Lab and 8.98 µl ddH<sub>2</sub>O. The DNA amplifications were performed with the following temperature profiles: 1 cycle of 3 minutes at 94°C for initial denaturing, 45 cycles of 1 minute at 94°C, 1 minute at 37°C, and 2 minutes at 72°C for denaturing, primer annealing and primer extension, respectively. The last cycle was followed by a final incubation for 10 minutes at 72°C. The amplified product was stored at 4°C until electrophoresis.

### 2.3.2. Gel Electrophoresis and Visualization

For separation of fragments, 9µl DNA amplified products pre-mixed with 2µl 6x loading buffer were loaded on 1.5% (w/v) agarose gels and electrophoresed in 1x TBE buffer (containing 10.8g of Tris, 5.5g of boric acid and 0.74g Na-EDTA) for 1.45 hours at constant voltage of 90V. A 100-basepair ladder (*ROTH*) was loaded on marginal lanes to estimate the size of the fragments. After electrophoresis, the gel was stained with ethidium bromide (10µg/ml) on a shaker containing 400 µl of distilled water for 30 minutes and then destained in distilled water for 20 minutes. The stained DNA was visualized UV transluminator (BioDocAnalyser™) and photographed by digital camera, connected to PC Bio Doc Analyse program and saved for later data scoring.

## 2.4. Data Scoring and Analysis

Each RAPD band was considered as a two-allele system, with only one of the alleles being amplifiable by the PCR. Data were scored as 1 for the presence and 0 for the absence of a DNA band for each locus across the 48 genotypes. A locus is considered as polymorphic when the frequency of present allele or null allele is less than 95% across the whole

genotypes investigated.

Genetic diversity was calculated based on Nei's unbiased gene diversity [14],  $H_j(i) = 2q(1-q)$ , where  $q$  is the frequency of null allele at a locus, with modification provided by [15] using polymorphic loci only. Gene diversity for each population was calculated for every locus according to [15] as  $H_j(i) = 2q_j(i)[1 - q_j(i)] + 2Var[q_j(i)]$ , where  $q$  is the frequency of null allele at a locus for a given population and was calculated from  $x$ , which is the frequency of individuals within the population that lack the RAPD band, as  $q = x^{1/2}$

$\left[1 - \frac{Var(x)}{8x^2}\right]^{-1}$ .  $Var(q)$  and  $var(x)$  were calculated as  $(1 - x)/4N$  and  $x(1 - x)/N$ , respectively, where  $N$  is number of individual per population. The mean observed gene diversity within each population was then calculated as  $H_j = 1/L \sum_{i=1}^L H_j(i)$ , where  $L$  is the number of polymorphic loci.

Estimates of genetic differentiation between populations were calculated as  $G_{ST} = (H_T - H_S)/H_T$ , where  $H_S$  is the mean gene diversity per population averaged across all population for each polymorphic locus and  $H_T$  is the total gene diversity calculated from the overall frequency of the amplifiable and null allele for each polymorphic locus.  $F_{ST}$  was also calculated from Analysis of Molecular Variance (AMOVA; [16]).

Percentage of polymorphic loci for each population was estimated using POPGENE version 1.31 [17]. The extent of population differentiation ( $F_{ST}$ ) was determined from analysis of molecular variance using Arlequin version 2 [18]. For the estimation of genetic distances between populations, an unbiased genetic distance matrix [14] was generated using PopGene version 1.31. The Nei's unbiased genetic distance matrix was subjected to UPGMA (Unweighted Pair Group Methods with Arithmetic average) cluster analysis with NTSYSpc program [19]. Further, Nei's unbiased genetic distance matrix and geographic distance matrix were used in NTSYSpc program for the Mantel tests to determine if population level genetic distances and geographic distance were correlated.

## 3. Results

From an initial analysis of 7 Operon technologies (OPB and OPC series) RAPD primers using two randomly selected individuals from two populations, 4 primers were found detecting multiple bands per sample. The remaining 3 primers were either produced poor or no amplification products at all. These 4 primers of arbitrary nucleotide sequence were then used in this study to amplify DNA segments from the genomic DNA of 48 *P. africana* genotypes. All the 4 primers produced multiple and clear bands that had a degree of heterogeneity across the 48 individuals representing 6 natural *P. africana* populations. In some instances, certain individuals in a population failed to amplify. Where this was the case, the absence of bands was

coded as ‘unknown’. Fig. 2 shows the example of amplification products of primer OPB 15 which were resolved by agarose-gel electrophoresis and visualized by staining with ethidium bromide.

The 4 primers generated a total of 43 different bands across the 48 individuals. The size of the amplified fragments ranged from ≈ 300 to 1500 base pairs (bps) across the four primers. Thirty three of the 43 bands (76.3%) were polymorphic and used subsequently to evaluate genetic diversity in the populations. Ten bands, two from primer OPC 16, two from OPC 10, three from OPB 15 and three from OPB 8, were found to present in all specimens. When

averaged across primers, the mean number of amplified loci and percentage of polymorphic loci per primer were 11.3 (ranging from 9 to 15), and 9.3 (ranging from 6 to 14), respectively.

Only 23.7% of the bands were monomorphic across the six populations. The percentage of polymorphic loci, considering those loci with the frequency of the most common allele is ≤ 0.95, was considerably wide across populations, for a single population it ranged from 50% for a population from Bulki to 70.5% from Chilimo, with a mean of 62.2% across the four primers. The number and percentage of polymorphic RAPD bands for the 6 populations is presented in Table 3.

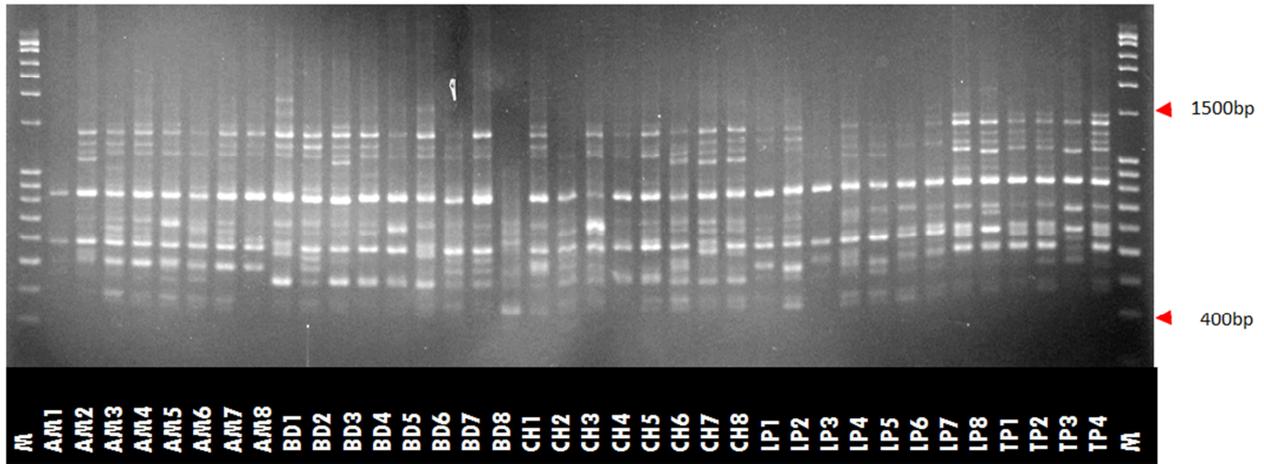


Figure 2. Photograph of ethidium bromide stained gel of RAPD fragments using primer OPB 15 DNA samples from Agere Mariam (AM), Bedele (BD), Chilimo (CH), Lepisi (LP), and Tepi (TP). Outside lanes (M) show an extended 100-bp ladder.

Table 2. Primer-wise Nei gene diversity estimates, partitioning of the genetic variation into within and between populations ( $G_{ST}$ ), and  $F_{ST}$  obtained from AMOVA.

Primer	Nei Gene Diversity estimate			AMOVA
	$H_s$	$H_T$	$G_{ST}$	
OPC 10	0.205	0.319	0.291	0.40
OPC 16	0.254	0.335	0.240	0.163
OPB 15	0.254	0.343	0.261	0.220
OPB 8	0.270	0.334	0.275	0.335
Mean	$\bar{H}_s$ 0.246*± 0.038	$\bar{H}_T$ 0.334*± 0.054	$\bar{G}_{ST}$ 0.264*± 0.055	$\bar{F}_{ST}$ 0.257*

Table 3. Number and percentage of polymorphic bands, and mean genetic diversity ( $H_w$ ) of the six populations of *P. africana* calculated from RAPD data using Nei unbiased gene diversity [14] with modification of Lynch and Milligan [15].

Population	Number of polymorphic bands	Percentage of polymorphic bands (%)	Mean $H_w$ + SE
Agere Mariam	20	58.8	0.182 ± 0.037
Bedele	19	55.8	0.175 ± 0.041
Chilimo	24	70.59	0.307 ± 0.038
Lepisi	23	67.65	0.297 ± 0.040
Tepi	24	70.59	0.290 ± 0.037
Bulki	17	50.0	0.150 ± 0.044
Mean	21.17	62.26	0.234

Estimation of the Nei gene diversity that was calculated for each population by using Nei unbiased [14] gene diversity with modification of Lynch and Milligan [15] as  $H_w$ , which is the average value across the whole loci, is given in Table 2 and 3. Pooled over the four primers, the diversity within each population ranged from 0.150 ± 0.044 for Bulki to 0.307 ± 0.037 for Chilimo, with greatest genetic diversity estimates in the larger populations (Chilimo, Lepisi

and Tepi) compared to the smaller populations (Agere Mariam, Bedele and Bulki). The average genetic diversity across the six population was found to be 0.234, indicating medium RAPD variation. Similarly, assuming Hardy-Weinberg equilibrium, the overall species genetic diversity calculated across the four primers as  $\bar{H}_T$  was found to be 0.320 (± 0.05). The overall within population genetic diversity estimated by as  $\bar{H}_s$ , which is the mean value

obtained by averaging per locus values across all loci, was found to be 0.236 ( $\pm 0.04$ ; Table 2).

Partitioning of the populations genetic variation showed that genetic diversity within populations,  $\overline{H}_s=0.236$ , accounted for 73.6% of the total genetic variation (Table 1). Genetic diversity among population accounted for 26.4%, indicating considerable differentiation among the populations of sampling. This result was reflected by  $G_{ST} = 0.264$ , which

measures the proportion of the genetic diversity attributable to population differentiation. Additionally, the analysis of molecular variance calculated using the present/absent data for the six populations' revealed significant genetic difference ( $P < 0.00001$ ) among the six populations *P. africana* (Table 4). Of the total genetic diversity, 27.9% was attributed to population differences and to 72.1% individual differences within populations.

**Table 4.** Analysis of molecular variance (AMOVA) calculated for the six populations of *P. africana* based on only polymorphic loci.

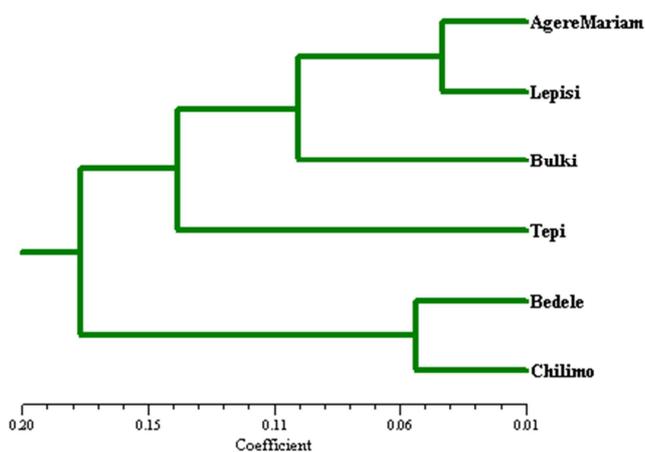
Source of variation	um of d.f.	Variance squares	Percentage components	Fixation of variation	Index
Among populations	5	65.44	1.360 Va	27.9%	FST: 0.279
Within populations	42	148.44	3.520 Vb	72.1%	
Total	47	213.88	4.880		

Nei's genetic distance calculated between pair of populations was lowest (0.061) between Agere Mariam and Lepisi, while the highest (0.213) was found between Chilimo and Agere Mariam (Table 5). The average genetic distance was 0.147, indicating the existence of considerable level of genetic differentiation among the populations. Genetic relationships among populations were further examined by UPGMA clustering analysis and represented by a dendrogram (Fig. 3). The genetic distance estimate between populations was tested for its goodness of fit for cluster analysis. The co-phenetic correlation between the genetic distance matrix and its co-phenetic value matrix was found to be 0.889, which indicates that its goodness of fit for cluster analysis is good, as described in [19].

The resulting dendrogram separates the six populations into two primary clusters which somewhat reflects their geographic locations. The first cluster includes the southern populations (Agere Mariam, Lepisi, Bulki and Tepi) with the Tepi material separated first from the three populations by a genetic distance coefficient of 0.044, and then the Bulki material separated from the Agere Mariam and Lepisi populations by 0.04 genetic distances. The second cluster includes the Western populations (Bedele and Chilimo), that appears to be separated from the first cluster with 0.051 genetic distance. The mantel test computed using the genetic distance matrix among populations and geographical distances matrix between these populations revealed a positive, but weak correlation between these variables, suggesting only marginal isolation by distance in these populations. ( $r=0.321$ ;  $P < 0.003$ ).

**Table 5.** Nei's (1978) Unbiased Measures of Genetic distance among the six populations.

Population	Agere Mariam	Bedele	Chilimo	Lepisi	Tepi	Bulki
Agere Mariam	0.000					
Bedele	0.173	0.000				
Chilimo	0.213	0.081	0.000			
Lepisi	0.061	0.148	0.210	0.000		
Tepi	0.140	0.150	0.196	0.183	0.000	
Bulki	0.096	0.125	0.204	0.100	0.123	0.000



**Figure 3.** Dendrogram based on Nei's (1978) genetic distances between the six populations of *Prunus africana*.

## 4. Discussion

The application of four RAPD primers to 48 individuals of *P. africana* detected high percentage of polymorphic loci (76.7%) across the whole material, and wide range of percentage of polymorphic loci for populations investigated. The polymorphism detected as percent of polymorphic loci in this study was comparable to the 80% reported by [12] using RAPD marker for analysis of molecular genetic polymorphism in *Sesbania sesban*. The present estimates suggest the existence of high genetic polymorphism in *P. africana*, and the use of RAPD technique to detect genetic polymorphism, at least for this species, is powerful.

The extent of overall within genetic diversity observed in this study can be only loosely compared with other long-lived species RAPD studies due to differences in the number and range of individuals surveyed, number and type of primers used, and life-history traits. Nevertheless, the value observed for *P. africana* is not particularly low to the mean value of 37 RAPD studies (0.25) on long-lived perennials in a review by Neybom [20]. The mean molecular variation reported in this review is probably somehow underestimated

because sexually as well as clonally reproducing species were included.

The level of variation observed within each of the studied populations was, however, quite different in such a way that the overall species genetic variation is maintained only by some populations. Slightly more than two fold of difference in the extent of genetic diversity was found between populations. Population size may be critical for the maintenance of genetic variation [21]. In large populations, genetic drift is insignificant, but it becomes important in small populations and may be particularly pronounced after dramatic reduction in range size and fragmentation of habitats of a species.

Highland forests in Ethiopia has long been threatened by anthropogenic induced deforestation and habitat fragmentation that begins approximately in 18<sup>th</sup> century and has accelerated strongly since then [22]. Besides, in areas like Bedele and Ageremariam, natural populations of *P. africana* and many other valuable species have also threatened by excessive logging and illegal tree felling (attributed to somewhat free access). Only few trees on patchy natural forests and other land use systems are indeed found in these regions. The lower level of variation detected for these populations could therefore be due to the pronounced effect of genetic drift in these populations owing to the smaller number of breeders that may contribute to the next generation. On the other hand, low level of anthropogenic pressure (attributed to low human population size) in Tepi and Chilimo areas seems to help *P. africana* populations in these areas to retain large population sizes and hence better genetic diversity.

Estimation of population differentiation based on  $\overline{G}_{ST}$  and  $F_{ST}$  in the present study was revealed more or less similar result. This is in lined with [20] who indicated that  $\overline{G}_{ST}$  and  $F_{ST}$  obtained by AMOVA usually produce very similar estimates when applied to the same plant material using the same set of marker data.

The relative level of genetic variation partition detected between *P. africana* populations may be explained by the particular life history trait of the species, which is long-lived, out-crossing, animal-mediated mode of pollen and seed dispersal, and mid to late successional stage [23] - all of which are factors that may promote low genetic differentiation between populations [20]. However, at the spatial scale of population sampling, the present data is relatively higher to those reported previously among natural population of *Prunus africana* in Cameron (11%) [24] and for other *Prunus* species ([25] in *P. mahaleb*). Furthermore, the result of this study is well above when compared with figures reported for other out-crossing tree species sampled on a similar geographical scale, which generally indicates a low level of partitioning among populations [26], even in species with disjunct and restricted distribution [27].

Considering the geographical feature that appear separating the studied populations, the among population split revealed from this study can, however, regarded as low.

The studied populations are found in East (Ageremariam and Lepisi) and West (Chilimo, Bedele, Tepi and Bulki) side of Ethiopian Rift Valley (ERV), which is identified as a major barrier of gene flow between population groups [28]. [24] reported 58.6% among population variation between Western and Central populations of *P. africana* in Kenya that are separated by Eastern rift valley. The level of variation and the pattern of phenogram (clustering of Tepi and Bulki populations with the Eastern populations) in the present data however indicate the existence of genetic connection between both sides of population over this barrier. This could be explained by ancient migration events (range expansion in moist periods followed by contractions into higher altitude as the climate warms) of *P. africana* that takes place over many Mellenia.

The evolution and current wide distribution of *P. africana* are believed to be shaped by long distance seed dispersal events and ancient overland migrations following past climatic fluctuations [29]. During interglacial periods (when the weather was moist and cool) *P. africana* (montane forests) may have descended to lower altitudes, providing connectivity between mountain systems [29, 30]. Such migration (range expansion) events could also have takes place, as presumed by [31], down to the bottom of Ethiopian Rift Valley that might probably provided suitable growing conditions for the species. These migrated individuals could, therefore, act as 'stepping stone' between populations of *P. africana* which are capable of long distance seed dispersal [29]. The pattern documented in this study can, therefore, be explained by this historical phenomenon.

On the other hand, the extent of among population split documented in this study can be regarded as very high when considering dispersal mechanism of *P. africana*. *P. africana* is an insect pollinated tree species with birds and primates mediated seed dispersal mechanism [32]. These dispersal agents, especially primates are capable of transport seeds over considerably long distances by ingestion [29, 32] and thereby keep populations genetically connected. Low level of  $F_{ST}$  is therefore expected between its populations. The value obtained in the present study was however well above the average  $F_{ST}$  value of 9% obtained for animal dispersed, out-crossing long lived species [33] and that of  $G_{ST} = 0.05$  reported for animal dispersed tropical woody species [34]. These results are evidences for the existence of thorough seed dispersal between populations that contributes to low genetic differentiation. Reproductive isolation or recruitment limitation can explain this result, although it was also found that significant genetic structure suggesting isolation by distance.

As indicated earlier, the studied populations exist at different elevations (ranging from 1600 m.a.s.l. (Tepi) to 2420 m.a.s.l. (Bulki), and thus may have different flowering and fruiting periods. For insect-pollinated and animal dispersed species such as *P. africana*, this difference in phenol phases combined with their very short anthesis periods [35] might result reproductive isolation of populations. Because, insect-mediated pollen flow can reach potentially long distances,

whenever reproductive individuals in anthesis are coincident temporally [36]. The low probability of gene flow between populations due to their reproductive isolations could therefore be the reason for the patterns of genetic variation distribution documented for *P. africana* in this study.

Gene flow between *P. africana* populations through animal mediated seed dispersal could be high as animals are capable long distance seed dispersal [29]. However, factors involved in vicariance events could have an important effect in populations of long-lived tree species [37]. Clumps of related seedlings could arise from seed caches, but the implications for genetic structure will depend on seed and/or seedling mortality prior to reproduction. Even if fruiting periods of two populations occur, demographic bottlenecks acting during the recruitment stages that follow seed dispersal can cause disproportionately high mortality of dispersed seeds, established seedlings, and saplings [38] and, as it was reported for other *Prunus* species [25], and many other tropical tree species [38, 39], this can also be the case *P. africana*.

There is evidence that establishment of fleshy fruited species with adaptation to endozoochorous seed dispersal like *P. africana* could be more limited by recruitment than by the availability of seeds [40]. Previous studies have also supported this view by reporting low level of population differentiation among animal dispersed species when compared to other life history trait [25, 41]. Thus, though additional evidences are required, such higher level of genetic differentiation detected in the present study may be primarily due to disproportional post dispersal mortality. Additional studies could elucidate whether this higher level of differentiation is caused by reproductive isolation or recruitment limitation.

## 5. Implication for Conservation

The significant levels of genetic differentiation observed in this study suggest that both populations should be considered as separate unit for the purpose of conservation of *P. africana* genetic resource. The recommendation here is that as many populations as possible should be preserved. Conserving all of the populations may help to capture the ecological and geographic range of the species. Moreover, the remaining populations of the species are few and some of them are already highly reduced in number of individuals. Conserving all remaining populations would reduce the risk of the species in those regions to become extinct as the result of environmental stochasticity [42]. As populations are genetically significantly differentiated, it would also preserve a large evolutionary potential if all populations are preserved.

Comparatively genetic differentiation documented in this study suggests that human induced seed dispersal from one location to another will result hybridization that may erode genetic integrity and conservation of local genetic resources. Conservation efforts must therefore consider the impacts of future afforestation program particularly if planting of *P. africana* from one locality is encouraged in the different

locality. Although the effects of intra specific hybridization in *P. africana* are unknown, high differentiation suggests hybridization may have a significant impact on long term fitness [12].

The lower genetic diversity of Bulki and Bedele populations compared to others may have implications for genetic management. This may indicate the need of conservation measures to be taken primarily to ensure future survival of the species. Because, while the relationship between low heterozygosity and fitness is complex and not yet clearly predictable [43], some studies show that reduced heterozygosity can cause a decrease in fitness [44, 45]. Conservation effort should focus on maintaining large populations in order to counteract the potential negative effects of drift and promote the maintenance of genetic diversity in these populations. Thereby, conservation effort might improve the population fitness in the short-term and population sustainability in the long-term.

With limited resources that may be available for developing conservation programs, if populations are to be ranked, emphasis should be on populations with highest amount of polymorphic markers, assuming a marker fragment represents unique genetic variation that is important for long-term evolutionary potential. One criterion used to classify a specific population as a genetic resource for conservation is its distinctness within a species. The decision to preserve a certain population as a genetic resource should be drawn from the determination of the mean genetic distance of this population to many other populations of the species [46].

Applying this to the analysis carried out here one would classify the following populations as important genetic resources: Chilimo 0.181, Tepi 0.158, and Lepisi 0.141. The present study also indicated that these populations are relatively rich in genetic diversity. Hence, the Chilimo, Tepi and Lepisi populations need to get focus for conservation. Apart from high levels of marker polymorphism, when other aspects of the studied populations is considered, i.e. size of the population and regeneration status, the Chilimo, Lepisi and Tepi populations carry larger population size (many individuals) with relatively different age structure, i.e. seedlings and juvenile, and mature individuals (as it was observed during field data collection). For *in situ* conservation, it is essential if there are as many trees as possible that produce seed, resulting in a new generation that is genetically diverse and able to adapt to changes in the environment [46]. Furthermore, trees on these areas occur relatively within a protected natural forest. Therefore, to insure that a maximum of the genetic variation detected in these populations will be available for future reforestation or other uses in the country and elsewhere, these populations need to be given priority for *in situ* conservation.

*Ex-situ* conservation effort through establishment of field genes banks should also begin attention to these populations with higher variability. However, taking the significant genetic structuring ( $F_{ST}$ ) between the studied populations into consideration, which is an indication of the

presence of unique individuals with unique genes in these populations, it is recommended that *ex-situ* conservation effort of *P. africana* should consider all populations. More particularly, for Agere Mariam, Bedele and Bulki populations, which left very few numbers of individuals in a very scattered manner on other land use types, implementing *ex-situ* conservation measures is the most appropriate solution to preserve their germplasm.

## 6. Conclusions

Generally, the level of RAPD variation detected for overall species diversity and within populations in this study suggesting the need to look for ways of maintaining and improving this variability. Partitioning of the total genetic diversity into its components (both by  $\overline{G}_{ST}$  and  $F_{ST}$ ) revealed that much of the genetic variation ( $\sim 72\%$ ) is found within populations. This might result from the high level of genetic variability maintained by the life history characteristics of the species. Although differentiation among these populations is not as such very high, it is comparatively higher than past results obtained for animal dispersed species, which may be explained by reproductive isolation or recruitment limitation. Future studies can identify whether this higher level of differentiation is caused by reproductive isolation or recruitment limitation. The result implies the need of considering each population as separate entities for conservation purpose.

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