EVIDENCE OF GENETIC DIVERSITY AND TAXONOMIC DIFFERENTIATION AMONG *ACACIA SENEGAL* POPULATIONS AND VARIETIES IN KENYA BASED ON RANDOMLY AMPLIFIED POLYMORPHIC DNA MOLECULAR MARKERS

S.F. Omondi

Kenya Forestry Research Institute, P.O Box 20412-00200, Nairobi

ABSTRACT

Acacia senegal is a multipurpose tree species that forms an essential component of many farming systems in Sub-Saharan Africa where it is commercially exploited for gum arabic production. However, the species is yet to be put to optimal production in some countries due to inadequate information on its population genetics and taxonomic delimitation. This study reports the use of 13 randomly amplified polymorphic DNA (RAPD) markers to determine genetic diversity and taxonomic relationships among 12 natural populations of *A. senegal* in Kenya. High genetic diversity was found for all populations. Mean gene diversity (He) for all populations was at 0.288 with effective number of alleles per locus (Ne) of 1.496. Analysis of molecular variance (AMOVA) revealed most genetic variations residing within (60%) rather than among populations. However, significant differentiation was detected among populations (ϕst = 0.130; $P < 0.001$). Cluster analysis based on similarity coefficient delimited three main groups corresponding to the three putative varieties of *A. senegal* namely *senegal, kerensis* and *leiorhachis*. The RAPD technology suggested high genetic diversity within the species and taxonomically differentiated the three varieties, however, there was evidence of admixture among the varieties. For high quality gum production and better economic returns, gum arabic collections should be designed based on the varieties and their locations. Seed collections for tree improvement or conservation programmes should take into account the groups detected for quality controls.

Keywords: *Acacia senegal*, genetic diversity, polymorphism, taxonomy, gum arabic, Semi-arid.

*Corresponding author:stephenf.omondi@gmail.com

INTRODUCTION

Arid and semi-arid lands (ASALs) covers more than half of African continent (Wickens *et al*., 1996). The ASALs are characterized by low annual rainfall, high temperatures and low soil fertility leading to frequent famines (Mark, 1997). However, these ecosystems sustain more than 75% of the sub-Saharan Africa human population. Despite the low vegetation cover, forest destructions go unabated in these areas (Wickens *et al*., 1996). Global studies show that destruction of tropical forests worldwide has increased dramatically in recent decades (Bawa and Seidler, 1998), posing a significant threat to biodiversity and biological processes in the drylands (Young *et al*., 1996). In Kenya, ASALs cover over 80% of the total land surface and support about 10 million people. These ecosystems also account for more than 70% of the country's eco-tourism interests, 60% of the nation's livestock, and up to 75% of wildlife population (Government of Kenya, 2005). These ecosystems are endowed with rich diversity of plant and animal resources that inhabitants have used and marketed locally for generations (Chikamai and Odera, 2002). Despite the abundant biodiversity, ASALs house many under-utilized tree species with great potential of improving living standards of the rural poor and revolutionizing the dryland economy. Among such species is *A. senegal,* which is the source of gum arabic, an internationally traded commodity (Chikamai and Banks, 1993).

Acacia senegal, also known as gum arabic tree, is a multipurpose tree belonging to the family Mimosoidae subgenus, *Aculeiferum* (Arce and Banks, 2001; Maundu *et al.*, 1999; Brenan, 1983). Taxonomically, the species has been identified to form four varieties (*kerensis*, *senegal*, *rostrata* and *leiorhachis*) with three of them (*kerensis*, *leiorhachis* and *senegal*) found in Kenya, sometimes closely distributed (Fagg and Allison, 2004). Generally, the species is commonly found in tropical and sub-tropical regions, South Africa and northwards to sub-

Saharan Africa and some parts of Asia (Raddad *et al.*, 2005). In Kenya, the tree is found in the northern, eastern, rift valley and coastal regions, mainly in the dry *Acacia* - Commiphora bushland (Chiveu *et al.,* 2008). The species is highly demanded because of its multiple uses such as; source of food, traditional medicine and pharmaceuticals, preservation and improvement of soil fertility, rites and customs, in addition to gum arabic which is the key product (Okunomo and Bosah, 2007; Luvanda *et al.,* 2006; Obua *et al.*, 2006;).

Research shows that the Kenyan gum arabic meets international standards. However, challenges in collection and maintaining high quality is hindering its smooth trade (Chikamai and Odera, 2002; Chikamai and Banks, 1993;). Other challenges include the significant variation in biochemical characteristics that has been found across the gum arabic collection range (Anderson and Weiping, 1992; Chikamai and Banks, 1993; Chikamai *et al*., 1996). However, the reported variation on gum arabic biochemistry can be used as a source of material for quality improvement through selection. To establish and manage the species for improved gum arabic production, selections, breeding and multiplication of elite individuals with high quality characteristics is required. Such improvement can be achieved through knowledge and understanding of the available genetic diversity and how it is structured.

Some information on genetic diversity of Kenyan population of *A. senegal* is available, however, these are not sufficient and none has addressed the taxonomic challenge of the species varieties. For example, a study by (Chiveu *et al*. (2008) using randomised amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers showed high genetic diversity with no differentiation among populations. This study did not analyse the whole range of the species distribution in the country. Another study by (Omondi *et al*. 2010) using simple sequence repeats (SSR or microsatellite) also recorded high genetic diversity within the species, however, this study was limited to variety *kerensis* alone. To develop a reliable improvement programme for *A. senegal*, elaborate genetic diversity study that captures the three putative varieties and the whole species distribution range is necessary. This will reveal how the three varieties interact and how this could affect gum production (Chikamai and Banks, 1993).

The aim of this study was to assess the level of genetic diversity and determine taxonomic differentiations among *A. senegal* populations in Kenya. Implications of the findings on management and improvement strategy of the species genetic resources are discussed.

MATERIALS AND METHODS

Population Sampling

Three hundred and sixty individual trees of *A. senegal* representing the three putative varieties (*kerensis, senegal* and *leiorhachis*) were sampled from 12 natural populations in Kenya (Table I). Healthy and clean leaf tissues were collected randomly from 30 adult trees per population at a distance of between 150 and 600 m apart depending on the size of the population and distribution of trees within the population. The leaf samples were dried in silica gel and stored at -20°C until DNA extraction.

TABLE I- LOCATIONS OF THE 12 POPULATIONS OF *A. SENEGAL* IN KENYA.

Population	Latitude	Longitude
Archers-post	00°39′52.7″	037°38′47.0″
Ngarendare	00°33′39.9″	037°20′45.3″
Daaba	00°32′00.2″	037°45′39.9″
Ntumburi	00º11’29.9”	037º30’46.7”
Kulamawe	00º33’32.8”	038º01’38.6”
Kajiado	02°02′59.5″	036°47′48.8″
Kibwezi	02°12’14.2″	038°03′17.4″
Magadi	01°32′04.3″	036°33′45.5″
Taita	03°27′01.1″	038°28′39.5″
Rimoi	00º39’52.5″	035º34’16.4”
Marigat	00°28′20.4″	035°55′10.6″
Koriema	00º26’12.5″	035º52’02.9”

DNA extraction and PCR amplification

Genomic DNA was isolated from leaves following a modified CTAB procedure (Fernández *et al*., 2000). DNA was quantified through comparison with low DNA mass ladder (Invitrogen) stained in ethidium bromide-stained 2% agarose gels. Forty random primers were screened for amplification using 30 DNA samples from across the populations. Thirteen of the primers showed clear and analysable bands. Polymerase chain reaction (PCR) amplification was performed using the 13 primers in a 25µl reaction volume containing 1XPCR buffer (10 mM Tris-HCL pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200mM each

of dNTPs, 20 µM of primer, 0.5 units of Taq-polymerase (Invitrogen) and about 25ng DNA template. The PCR amplification was performed using TECHNE TC - 412 Thermal Cycler (UK), with an initial denaturation at 94.5º C for 5 min., followed by 40 cycles. Each cycle consisted of denaturation at 94º C for 45s, primer annealing at 37ºC for 1 min, and extension at 72ºC for 2 min with a final extension at 72º C for 5 min. PCR products were separated on 1.5% agarose gel in 0.5 X TBE (Tris–Borate EDTA) buffer and stained in ethidium bromide (10mg/ml). The sizes of the amplified fragments were determined using a 100-base pairs (bp) DNA ladder (Invitrogen) run along the sides of the amplified products. The amplified products were visualized under ultraviolet light and photographed using Gel LOGIC 200 imaging system (Kodak MI SE).

Statistical analysis

Amplified DNA fragments (RAPD profiles) were scored for each individual as discrete characters and transformed into binary matrix (1 = presence, 0 = absence) across all individuals from all populations and for each primer used. Table 2 shows chosen parameters for genetic diversity test. Percentage of polymorphic loci was calculated for each population. Shanon's diversity indexes (I) and expected heterozygosity (He) was also determined. Nei's unbiased genetic distance (*D*) was determined using POPGENE 1.32 software (Yeh *et al*., 1997). The Nei's genetic distance matrix was used to generate the phylogenetic tree using unweighted pair group arithmetic average (UPGMA) method in MEGA software (Tamura *et al*., 2007). The reliability of this phenogram was evaluated by bootstrapping the data matrix.

The hypothesis, that populations are differentiated because of isolation by distance, was tested by correlating Nei's unbiased genetic matrix against the geographical distance matrix. Spearman's rank correlation coefficient was calculated and significance determined with 10,000 permutations using Mantel procedure (Mantel, 1967) available in GenAlEx 6.4 software (Peakall and Smouse, 2006). To analyze the intra- and inter-population genetic variation, analysis of molecular variance (AMOVA) was performed using GenAlEx 6.4 software (Peakall and Smouse, 2006).

RESULTS

Genetic diversity

Among the 40 random primers screened, 13 produced unambiguous, polymorphic and reproducible fragments while the others resulted to, either no amplification or smeared profiles, which could not be interpreted. The 13 primers yielded 250 bands (loci) with 243 (97.2%) being polymorphic. The band sizes ranged from 150 to 1500 bp. The number of amplified fragments per RAPD primer ranged from 15 (KFP 34) to 22 (KFP 10 and KFP 30) with an average of 19 bands per primer (Table III). This set of loci is expected to give a good sampling of the total genome and a good assessment of the genetic diversity. The typical example of the polymorphism detected with primer KFP 8 is as shown (Figure 1).

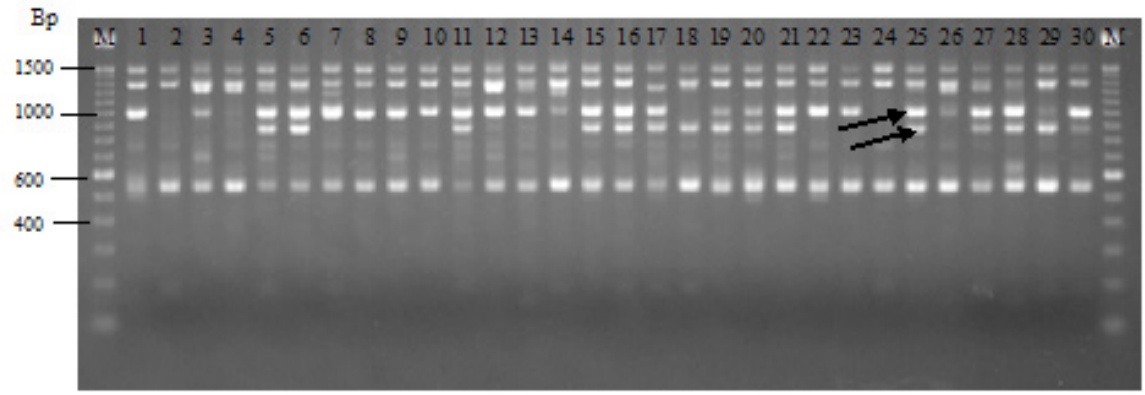

Figure 1. DNA banding profiles of 30 *Acacia senegal* samples from Daaba population using KFP 8 primer; arrows show polymorphic bands.

Distribution of total RAPD bands among populations appear to be highly constant (191-230 bands). Overall, the level of genetic diversity as shown by Shanon's diversity index (I) was relatively high varying from 0.360 (Marigat) to 0.485 (Daaba) with mean of 0.428. Based on mean percentage polymorphism (% P), diversity ranged from 67.2% (Magadi) to 90% (Ntumburi) with the overall mean of 80.8%. Expected heterozygosity (He) ranged from 0.234 (Marigat) to 0.329 (Daaba). Effective number of alleles (Ne) ranged from 1.456 (Magadi) to 1.570 (Daaba) with a mean of 1.496 (Table II). When the samples were considered putatively as varieties, the following diversity indices were observed; Ne (1.508, 1.495 and 1.477), I (0.441, 0.430 and 0.394), He (0.296, 0.288 and 0.268) and % P (83.5, 81.9 and 72) for variety *senegal*, *kerensis* and *leiorhachis,* respectively. Very few bands were unique to a single population. The 13 primers detected only 2 unique bands in 2 populations, one from Kibwezi and the other in Koriema.

TABLE II- GENETIC DIVERSITY INDICES OF THE 12 POPULATIONS OF A. SENEGAL IN KENYA.

Population	**N**	**Na**	**Ne**	**I**	**He**	**% P**
Archers-post	30	1.564	1.475	0.403	0.272	75.6
Ngarendare	30	1.660	1.531	0.444	0.302	79.6
Daaba	30	1.776	1.570	0.485	0.329	88.0
Ntumburi	30	1.820	1.531	0.465	0.310	90.0
Kulamawe	30	1.680	1.497	0.414	0.281	76.8
Kajiado	30	1.692	1.487	0.425	0.283	84.0
Kibwezi	30	1.716	1.545	0.462	0.313	84.8
Magadi	30	1.556	1.456	0.374	0.255	67.2
Taita	30	1.760	1.470	0.427	0.281	86.8
Rimoi	30	1.640	1.470	0.404	0.273	73.6
Marigat	30	1.528	1.379	0.360	0.234	76.4
Koriema	30	1.728	1.545	0.472	0.318	86.4
Overall mean	30	1.677	1.496	0.428	0.288	80.8

N-sample size, Na-Number of alleles per locus, Ne-Number of effective alleles per locus I-Shannon's diversity index, He-expected heterozygosity, % P-Percentage polymorphism

TABLE III- CHARACTERISTICS OF RANDOM OLIGONUCLEOTIDE PRIMERS USED IN THE STUDY

Primer	**Sequence**	**%GC**	**NB**	**%P**	**I**	**He**
KFP 8	ACGCGCTGGT	70	21	98.6	0.571	0.356
KFP 10	ACGGTGCGCC	80	22	98.6	0.61	0.389
KFP 22	TACGCACACC	60	20	98.9	0.595	0.397
KFP 23	GCTCGTCAAC	60	17	92.8	0.552	0.376
KFP 25	ACTCGTAGCC	60	21	96.1	0.498	0.363
KFP 30	GTGCGGACAG	70	22	98.3	0.592	0.410
KFP 34	GTCCGTGCAA	60	15	98.3	0.56	0.401
KFP 35	CGTAGCCCCG	80	21	98.8	0.535	0.385
KFP 42	GGTCGGAGAA	60	18	96.1	0.568	0.381
KFP 45	GGAAGTCGCC	70	21	96.9	0.584	0.383
KFP 46	AGTCGTCCCC	70	19	96.7	0.572	0.372
KFP 48	CTGCATCGTG	60	17	97.2	0.564	0.375
KFP 49	GAAACACCCC	60	16	96.1	0.577	0.377
Mean			**19**	**97.2**	**0.568**	**0.382**

*NB, number of bands

Genetic structure

In assessing relatedness of the populations, Nei's unbiased genetic distance (*D*) was used. The shortest distance was recorded between Kulamawe and Magadi (0.020) populations while the largest distance (0.349) was between Magadi and Koriema populations (Table IV). Analysis of molecular variance (AMOVA) revealed higher significant genetic variation within populations (60 %; $P < 0.001$) than among the populations (27 %; $P < 0.001$).

When the populations were grouped as varieties, 13% ($P < 0.001$) of the variance was attributed to the varietal differences. The AMOVA results is an indication of significant population differentiation as also shown by ϕst values (0.130; $P = 0.000$, 0.314; $P = 0.000$, 0.403; $P = 0.000$) for among varieties, populations and within populations respectively (Table V).

TABLE IV- UNBIASED NEI'S PAIRWISE GENETIC DISTANCE MATRIX BETWEEN POPULATIONS OF ACACIA SENEGAL IN KENYA.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	0.000											
2	0.177	0.000										
3	0.109	0.082	0.000									
4	0.332	0.184	0.288	0.000								
5	0.078	0.211	0.141	0.304	0.000							
6	0.349	0.201	0.304	0.020	0.311	0.000						
7	0.178	0.170	0.098	0.339	0.168	0.349	0.000					
8	0.111	0.174	0.162	0.275	0.060	0.287	0.182	0.000				
9	0.168	0.162	0.089	0.338	0.156	0.348	0.051	0.182	0.000			
10	0.209	0.284	0.273	0.292	0.237	0.305	0.304	0.288	0.297	0.000		
11	0.097	0.219	0.141	0.349	0.124	0.374	0.174	0.128	0.148	0.232	0.000	
12	0.188	0.115	0.207	0.182	0.214	0.186	0.279	0.165	0.283	0.257	0.205	0.000

*1-Koriema, 2-Ngarendare, 3-Daaba, 4-Kulamawe, 5-Ntumburi, 6-Magadi, 7-Kibwezi, 8-Kajiado, 9-Taita, 10-Rimoi, 11-Marigat, 12-Archers-Post

TABLE V- ANALYSIS OF MOLECULAR VARIANCE (AMOVA) OF 360 INDIVIDUALS OF A. SENEGAL TREES FROM TWELVE NATURAL POPULATIONS IN KENYA USING 13 RAPD PRIMERS. DF-DEGREES OF FREEDOM, ϕST-POPULATION DIFFERENTIATION

Source of variation	**DF**	**Sum of Squares**	**Estimated variance**	**% variation**	**ϕst-Value**	***P*-value**
Among varieties	2	2803.539	7.882	13	0.130	0.000
Among populations	9	4812.942	16.617	27	0.314	0.000
Within populations	348	12614.767	36.249	60	0.403	0.000
Total	359	20231.247	60.749	100		

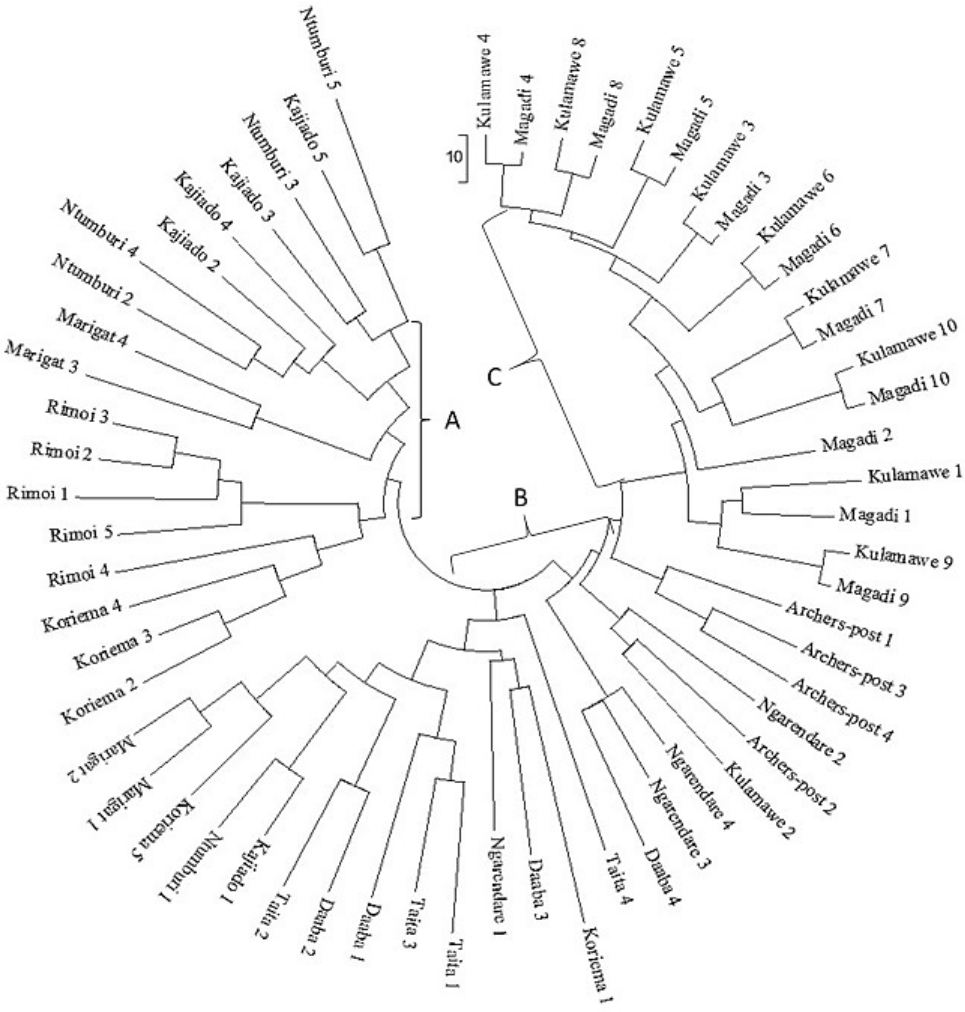

Figure 2. Cluster analysis of the 12 populations of *Acacia senegal* in Kenya showing the differentiation of the three varieties. A, variety *senegal*; B, variety *kerensis*; C, variety *leiorhachis*

DISCUSSIONS

Genetic diversity

Overall in the present study, Kenyan population of *A. senegal* has revealed high genetic diversity. Assessment of genetic variability among individuals and populations using RAPD markers is promising because many polymorphic loci can be obtained in a relatively shorter time inexpensively without any prior knowledge of the genome of the species under study (Chiveu *et al*., 2008). In the present study, RAPD analysis was found to be important and a powerful tool for detection of spatial genetic variation. With 13 random primers, 243 polymorphic loci were obtained and differentiated the 360 individuals of *A. senegal* studied reflecting a rich allelic diversity in the populations.

The high genetic diversity detected in this study reflected in allelic richness, percentage polymorphism and heterozygosity is important in sustainable use of the species genetic resources. The diversity found is comparable to those reported for the species by Chiveu *et al*. (2008) and other tropical species such as *Vitellaria paradoxa* (Bouvet *et al*., 2004), *Acacia melanoxylon* (Playford *et al*., 1993) and *Araucaria araucana* (Bekessy *et al*., 2002). The high values of genetic diversity may be attributed to life history of the species and its spatial distribution, and are in agreement with the general conclusions already made on other widespread, long lived and out-crossing insect

pollinated tree species.

Acacia senegal is widely distributed in the drylands and its adaptation to the varied environment requires high genetic diversity. Findings of the present study also corroborates high genetic diversity reported earlier by Chevallier *et al*. (1994) on the species using isoenzymes. They concluded that the significant heterozygosity observed in *A. senegal* and partitioning of higher percentage of genetic diversity within population suggests presence of exclusive out-crossing mating system. Such mating pattern promotes inter-breeding and genetic diversity. Additionally, high genetic diversity in tropical trees such as *A. senegal* may be attributed to high levels of gene flow among populations through seed and pollen movements (White *et al*., 2002). Other traits such as self-incompatibility has also been reported for *A. senegal* that may be ensuring diversity among individuals within populations (Doligez and Joly, 1997). Such variations are important in conservation biology and improvement programmes since they contain the future evolutionary adaptations and value addition opportunities (Shrestha *et al*., 2000).

Forest trees are non-mobile and long-lived organisms, which grow under environmental conditions that are heterogeneous in time and space. Moreover, they are exposed to many stress factors, most of which are due to human activities, pollution, climate change, habitat fragmentation amongst others (Whitmore, 1997). For survival, higher genetic diversity is required. In this study *A. senegal* has shown high adaptive and improvement potential revealed through its high genetic diversity needed to survive, persist over time and avail opportunities for selection. The species is highly valued for its gum arabic production and potential economic stay for arid areas. Therefore, higher diversity presents chance for improved and sustainable utilization. Among the populations studied, higher level of genetic diversity was observed among the widely dispersed populations while the more restricted such as Magadi population had the lowest level of variation. As has been reported in other studies, distribution range and population size has a major correlate of within population genetic variation in tropical tree species with restricted populations showing less variation than those with broader distribution (Loveless, 1992). This scenario explains the present findings.

Genetic structure and variety differentiation

Plant species differ markedly in the way genetic diversity is partitioned between populations. These patterns are correlated with mating systems and life history parameters (Hamrick, 1989). Distribution of variability between and within populations in the present study based on nested analysis of molecular variance indicates that most of the genetic variation is present within populations (60%). This result was expected with the biological characteristic of *A. senegal*. Hamrick *et al*. (1991) identified those characteristics of species, which can explain high level of genetic diversity within populations and low levels among populations. These include life history, dispersal mechanism, mating systems and distribution range. Species such as *A. senegal* that do not have strong habitat specificity and are continuously distributed are expected to have more within-populations diversity than those with strong habitat preference and a scattered distribution.

Association between breeding systems and levels of genetic diversity has been well documented. Generally, most selfing species are characterized by high genetic differentiation among population whereas predominantly out-crossing wind pollinated species exhibit less variation among populations (Loveless and Hamrick, 1984). *Acacias* are generally out-crossers (Ross, 1979; Oballa, 1993). However, significant variation was detected among populations of *A. senegal* in this study. This is contrary to the common belief about the out-crossing woody perennial plants. In this regard, the high degree of population differentiation realized here is unlikely to be a result of inbreeding. Pollination trials on *A. senegal* have shown that the species is exclusively out-crossed and self-incompatible (Tandon and Shivanna, 2001). Differentiation among populations of *A. senegal* could therefore be attributed to taxonomic difference within the species whereby four putative varieties (*rostrata*, *kerensis, senegal* and *leiorhachis*) have been recognized. Three of these varieties (*kerensis, senegal and leiorhachis*) are reported present in Kenya (Chikamai and Banks, 1993: Omondi *et al*., 2010) and may be the source of differentiation revealed in the present study. During sample collections, materials were collected from the whole species distribution range, possibly sampling all the three varieties. Inclusion of all the three varieties in this analysis might be the contributing factor for differentiation.

The distance based clustering analysis method revealed a strong structure among the 12 populations of *A. senegal*. Individuals were grouped into three groups reflecting the three putative varieties (*kerensis*, *senegal* and *leiorhachis*)

with several overlap between *kerensis* and *senegal*. Such findings have been reported in the taxonomic study of *A. senegal* in Uganda using morphological characters (Mulumba and Kakudidi, 2009). Field observations have also been made in Kenya where variety *senegal* and *kerensis* show morphological overlaps unlike the distinct *leiorhachis* (Omondi *et al*., unpublished data). The results agree with initial taxonomic work that separated the species into different varieties (Brenan, 1983). This finding lends support to the concept of keeping the varieties separate in utilization (Chikamai and Banks, 1993). Since the species has generated a lot of interest based on its potential for quality commercial gum arabic production in Kenya, there is need for caution to treat the varieties differently. Biochemical studies of Kenyan gum arabic have established quality variations and this could be attributed to the mixing of gum from the different varieties.

Ecological and geographic differentiations are important factors, which influence breeding and sampling strategies of tree crops. In the test of hypothesis of isolation by distance, results of this study found positive but non-significant correlation between genetic and geographic distances. Populations that are located far apart were found to be clustered together while those closely located separated. This is an indication of efficient gene flow among the populations. The overall pattern of genetic divergence among the populations studied reflects a story of short-term separation and consistent gene flow. The populations thus share gene pool, and there is no evidence of any barriers likely to restrict gene flow between them.

CONCLUSION

To improve on the quality of gum arabic production and good market returns, gum collections should be done and separated based on variety. Through this, livelihoods of the local populations that entirely depend on the resource will be enhanced. From conservation point of view and since varieties clustered separately, each variety should be conserved separately. A representative sample of natural populations of each variety could then be used to develop *in situ* or *ex situ* conservation strategy of the species. Findings could help define a strategy for elaborate breeding population. To start an improvement programme, the breeding population should consist of many individual trees selected within few populations of the varieties to capture larger proportion of the variation.

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