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Genetic diversity and population structure of three commercial indigenous *Aloe* species in selected ASALs of Kenya

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Abstract

Aloe species are common in arid and semi-arid lands (ASALs) of Kenya and are of economic importance especially for bitter gum production. However, the species is under ecological threat due to unsustainable harvesting from the wild. The objective of this study was to evaluate genetic diversity and population structure of three commercially exploited indigenous *Aloe* species; *A. secundiflora*, *A. turkanensis* and *A. scabrifolia*. The study was carried out in fifteen populations of *Aloe* species in their wild habitat where their geographic distribution was surveyed and populations delineated. Within each population, 30 trees were sampled at a distance of 100 m apart where leaf tissues were collected for DNA isolation and analysis using modified Cetyl trimethyl ammonium bromide (CTAB) method. Randomly amplified polymorphic DNA (RAPDs) and Inter-simple sequence repeats (ISSR) markers were used to determine the genetic diversity and population structure. Ten RAPD and seven ISSR discriminative primers with the highest value of genetic diversity were selected to genotype three indigenous *Aloe* species. The genetic similarity was performed using Popgene 1.3.1; hierarchical UPGMA cluster analysis and principal coordinate analysis were performed using Genalex 6.5. To map genetic structure and gene pools, both Structure and Structure Harvester statistical packages were used. From the results, *A. secundiflora* species was the most distributed in the surveyed ASALs. There was high genetic variation among and within the populations. The combined use of ISSR and RAPD revealed high variations among the populations as compared to when either of them used singly. Genetic variation was highest within *secundiflora* species and least within *turkanensis* species. Maralal *secundiflora* population had the highest genetic variation. The study exhibited a population genetic structure with three major clusters which corresponded to the three *Aloe* species with minimal reproductive crossing among them. It is therefore, recommended to undertake biophysical studies to determine the advantages of *A.secundiflora* that has over the other species. In addition, Maralal *sec* population should be conserved *ex-situ* because of its high genetic diversity while both ISSR and RAPD markers should be prioritized in *Aloe* genetic studies as they reveal high variation.

Keywords: Genetics, diversity, population, structure, indigenous, *Aloe*, species

Introduction

Kenya is known for its rich diversity of native *Aloe* species with 59 species being reported by [1]. The government of Kenya through the vision 2030 blue print, projects to curb food insecurity through sustainable management of dry lands and its genetic resources [2] which includes the *Aloe* species. The indigenous *Aloe* species are important non-wood plants with many economic and socio-cultural uses found in ASALs of Kenya which is home to more than 30% human population [3,4]. Due to their abundant socio-economic potential, the indigenous

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Aloe species in Kenya have been harvested by the local communities in the ASALs from their natural populations for many years for traditional medicinal use, cultural and aesthetic purposes [5]. The commercial exploitation of *Aloe* species in Kenya was first reported in the 1960s with only five species being exploited for bitter gum production i.e. *A. secundiflora, A. turkanesis, A. rivae, A. calidophila* and *A. scabrifolia* [6,7]. Climate change, unsustainable harvesting of plants and their products, introduction of exotic species and pollution has been the key to unprecedented change in biodiversity worldwide [8]. This unsustainable extraction from the wild causes threat to ecological balance and finally, may lead to complete loss of the species. This has raised concern locally and internationally on the level and impact of exploitation of wild populations and prompted a Presidential decree in 1986, banning commercial harvesting of *Aloe* species from their natural populations [9].

Genetic diversity is a key component to biodiversity analysis and therefore it's important to have knowledge of the distribution, genetic diversity, environment and relations among plant varieties to recognize gene pools, identify gaps in germplasm collections and develop effective conservation and management strategies [10]. There are morphological variations in some economically important *Aloe* species [11]. The DNA based molecular markers are free from any environmental modulations unlike the morphological markers [12] and hence provide an important tool to determine the genetic diversity of *Aloe* species. The RAPD (Randomly Amplified Polymorphic DNA) and ISSR (Inter-Simple Sequence Repeat) marker systems have been widely used in genetic diversity studies of different plant species and offer alternative for studying genetic variation in *Aloe* species.

To address these challenges facing *Aloe* utilization in Kenya, this study therefore, determined the geographical distribution of the three commercial indigenous *Aloe* species (*A. secundiflora, A. turkanensis,* and *A. scabrifolia)* from selected areas in ASALs of Kenya to establish their distinct populations. In addition, the study evaluated the molecular characterization of the three *Aloe* species, using RAPDs and ISSR markers and mapped their genetic pools and structures. The output of this study was to provide important information in identifying gene pools, gaps in germplasm collections and development of effective conservation and management strategies for *Aloe* plants.

Materials and methods

Description of the study area

The study was carried out in selected ASALs of Kenya where *Aloe* species grow naturally in the wild with commercial exploitation for bitter gum production as shown below (**Figure 1**). The sampling populations came from Gwasi, Isebania, Kajiado, Laikipia, Loima, Lokitaung, Maralal, Mwingi, Oropoi, Samburu, Sultan Hamud, Baringo and Taveta (**Figure 1**).

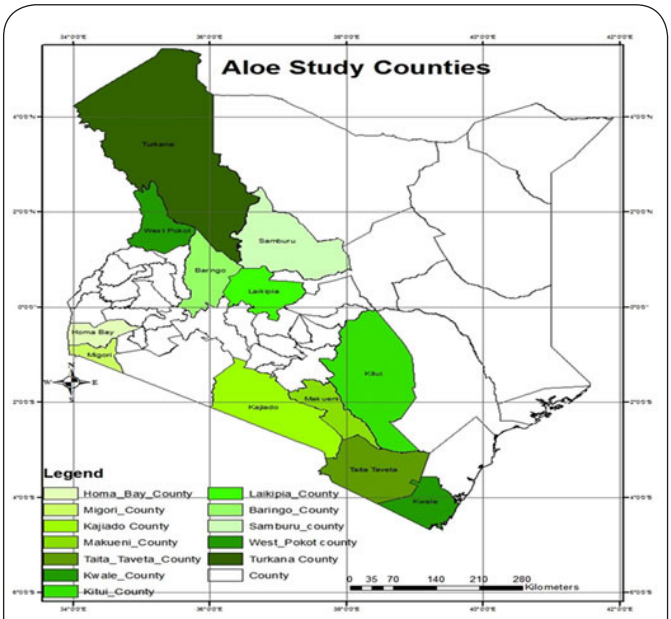

Figure 1. *Aloe* species distribution in the selected ASAL Counties of Kenya.

Sample collection from the field

Young and healthy leaf tissues of 450 individual *Aloe* plants within the 15 populations were randomly sampled with each population represented by 30 plants. A distance of about 100 m apart was observed between each plant to avoid picking genetically related individuals. The samples were preserved in silica gel for DNA isolation in the laboratory. The GPS positions of each sampled plants were also recorded and sites mapped.

DNA isolation and Polymerase Chain Reaction (PCR) analyses

The modified cetyl trimethyl ammonium bromide (CTAB) method [13,14] was used in this study. About 0.2 g of the dry leaf tissues were ground into fine powder using mixer mill (Retsch® MM400) and then transferred to a 2.0 ml microfuge tube containing 1,000µl isolation buffer (IB, 10 % polyethylene glycol, 0.35 M sorbitol, 0.1 M Tris-HCl-pH 8.0, and 0.5% β-mercapto-ethanol. The solution was mixed thoroughly by vortexing and then centrifuged at 10,000 rpm at 4 °C for 3 minutes then supernatant transferred to a new sterile 2 ml microfuge tube. Exactly 800 µl of isolation buffer (IB) was added and then vortexed to mix thoroughly and centrifuged at 10000 rpm at 4^0C for 3 minutes and supernatant removed. This step was repeated 2-3 times till the supernatant was less viscous. About 500 µl supernatant was taken and 500 µl CTAB buffer (1% CTAB, 0.05M Tris HCl, 0.7 M NaCl, 0.5% β-mercaptoethanol, 24:1 chloroform: Isoamyl alcohol, sorbitol, 0.1 M Tris-HCl-pH 8.0, and 10ng/µl RNAse) added and mixed by vortexing and then incubated at 65 °C for 60 minutes and another 30 minutes at 37^0C in oscillating water bath for another 60 minutes. Equal volume (800 µl) of CIA (chloroform isoamyl alcohol; 24:1 ratio) was added and mixed thoroughly by gentle inversion for 20 minutes and centrifuged at 14,000 rpm at room temperature

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for 10 minutes. 600µl of supernatant was then transferred to a new 1.5 ml microfuge tube and a tenth of 3M NaOAc (60µl NaOAc) and 600 µl isopropanol added. The solution was mixed by gentle inversion and centrifuged (15,000 rpm at 4°C) for 5 minutes. The supernatant was discarded and 800 µl of 70% ethanol added and flipped then centrifuged (15,000 rpm at 4°C) for 5 minutes to wash the DNA pellets. The supernatant was discarded and the DNA pellets air dried for 45 minutes and dissolved in 300µl DNase-free water. The dissolved DNA was then quantified using spectrophotometer (Biospec-Nano) and then uniformly diluted to 6.25µl ready for PCR analysis.

Polymerase Chain Reaction (PCR) analyses

The polymerase chain reaction method was used as described by [15]. Forty RAPD and twenty five ISSR primers were screened for polymorphisms. Ten RAPD and seven ISSR primers which gave distinct polymorphic amplified products were selected for subsequent analysis. The PCR analysis of 10 RAPD and 7 ISSR loci (**Table 1**) was performed in a final volume of 6.25 µl. Approximately 6.25 ng of template DNA was finally added in a 0.2ml 96 well PCR microplate (Thermo Scientific AB-600). The reactions master mix composing of 1x PCR buffer (DreamTaq buffer), 3.5mM $MgCl_2$ (Qiagen), 0.4mM dNTPs (Biolabs), 0.24µM primers, 2% PVP, 0.75U Taq polymerase (Dream Taq) and 6.25ng template DNA was added. All the PCR reaction preparations were performed on ice. The RAPD thermal cycling was then done with first cycle starting with an initial denaturation at 95°C for 10 minutes, then followed by 35 cycles of denaturation at 95^{0}C for 30 seconds, annealing at 37°C for 30 seconds and extension at 72°C for 1 minute. A final extension at 72^{0}C was performed to make sure everything had polymerized. The ISSR thermal cycling was done with first cycle starting with an initial denaturation at 95°C for 10 minutes, then followed by 35 cycles of denaturation at 95^{0}C for 30 seconds, annealing at 47.5°C for 30 seconds and extension at 72°C for 1 minute.

A final extension at 72^{0}C was performed to make sure everything had polymerized. The reactions were performed using Verity 96 thermo cycler (Applied Biosystems).

Gel electrophoresis

The gel electrophoresis method used was as described by [16,17]. Amplified fragments were separated using 1.4% agarose gel electrophoresis for 70 minutes. The gel was stained by adding 3.5µl SYBR Safe dye (Invitrogen 10,000x concentrate in DMSO) and molten in a microwave oven for 1 minute. DNA fragments were then mixed with 1x gel loading dye (Thermo Scientific) and then all 7.5µl were loaded into the gel well with the first and the last wells loaded with 100bp Plus DNA ladder (Thermo Scientific) for sizing the fragments. The gels were then viewed under UV illumination (ATTA E-Graph) at 320nm and photographs taken.

Data collection and statistical analysis

For each RAPD and ISSR primer, only the stained bands which could be unambiguously scored were used in the analysis. The number of polymorphic and monomorphic fragments for each primer pair were visually scored (for band presence (1) or absence (0)) and set in a binary matrix. The binary matrix data file created was then configured as an input file for data analysis. The percentage of polymorphic loci (P), polymorphic information content (PIC), Nei's genetic diversity index (H), coefficient of differentiation (Gst) were derived using PopGene

Table 1. ***Aloe*** **species RAPD and ISSR primer oligonucleotide sequences and Polymorphic Information Content.**

Primer code	Primer sequence 5'→3'	Type	Total bands	polymorphic bands	% of polymorphic bands	Total fragments	Polymorphic fragments	% of polymorphic fragments
UBC-808	AGAGAGAGAGAGAGAGC	ISSR	9	8	88.89	1297	847	**65.30**
UBC-809	AGAGAGAGAGAGAGAGG	ISSR	10	9	90.00	2287	1837	80.32
UBC-811	GAGAGAGAGAGAGAGAC	ISSR	8	8	100.00	2197	2197	**100.00**
UBC-827	ACACACACACACACACG	ISSR	7	6	85.71	2274	1824	80.21
UBC-861	ACCACCACCACCACCACC	ISSR	11	11	100.00	3549	3549	**100.00**
UBC-873	GACAGACAGACAGACA	ISSR	13	13	100.00	4895	4895	**100.00**
UBC-880	GGAGAGGAGAGGAGA	ISSR	13	12	92.31	5517	5067	91.84
KFP-3	GTT AGC GGC G	RAPD	13	12	**92.31**	3966	3516	**88.65**
KFP-10	ACG GTG CGC C	RAPD	15	9	60.00	5839	3139	53.76
KFP-8	ACG CGC TGG T	RAPD	11	5	45.45	4095	1395	**34.07**
KFP-17	CCG AAG CCC T	RAPD	16	11	68.75	5791	3541	61.15
KFP-21	GTA GGC GTC G	RAPD	9	4	**44.44**	3552	1302	36.66
KFP23	GCT CGT CAA C	RAPD	11	7	63.64	3835	2035	53.06
KFP-25	CTA GGC GTC G	RAPD	6	4	66.67	2076	1176	56.65
KFP-27	TCC TCG CGG C	RAPD	11	7	63.64	4102	2302	56.12
KFP-28	AAT CGG GCT G	RAPD	8	7	87.50	2378	1928	81.08
KFP-30	GTG CGG ACA G	RAPD	7	4	57.14	2102	752	35.78

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version 1.3.1 [18]. The analysis of molecular variance (AMOVA) and principal coordinate analysis were done using GenALEx software version 6.5 [19].

Mapping of the genetic pools of the three *Aloe* species

To map the population genetic structure of the three *Aloe* species (*turkanensis, scabrifolia* and *secundiflora*), the combined ISSR and RAPD genetic binary data were used to estimate the exact number of subpopulations on the basis of the maximum assumed or estimated populations (ΔK) values. To outline the major gene pools, analyses were performed using admixture model assumptions with correlated alleles; K was presumed to be 2–10, selected after 10 independent runs. Each run consisted of a burn-in period of 5,000 steps followed by 500,000 MCMC replicates [20]. The Structure Harvester statistical software [21] was used to collate the results obtained from Structure statistical software following [22] and maximum value of ΔK associated with each K value were analyzed to identify the number of clusters that best described the data.

Results and Discussion

Geographical distribution of *A. secundiflora, A. turkanensis*, and *A. scabrifolia* from selected ASALs of Kenya and their distinct populations

The distributions of *A. secundiflora*, *A. turkanensis* and *A. scabrifolia* in the studied ASALs of Kenya were distinct. *A. secundiflora* was dominant in all of the surveyed sites i.e. Coast, Lake, Northern and Central regions. *A. turkanensis* and *A. scabrifolia* were only reported in Northern Kenya (**Figure 2**).

The distribution of *Aloe secundiflora* in Central, Coast and the lake Victora regions was mainly found in virgin uncultivated bush-lands and rocky hills, dominated by *Acacia* spp, *Lantana camara*, *Agave* spp, and other native *Aloe* spp under rocky loamy soils. However, in the Northern region, the distribution was mainly in rocky conserved forested hilly areas and bushy grasslands dominated by *Acacia* spp, *Prosopis juliflora*, *Agave* spp, *Balanites aegyptica* and other *Aloe* spp. The soils in these areas were mostly rocky and stony loams.The *Aloe turkanensis* was found mainly in the coastal belt of Kenya inhabiting mostly conserved forested lands dominated by *Acacia* spp, *Prosopis juliflora* and *Agave* spp.

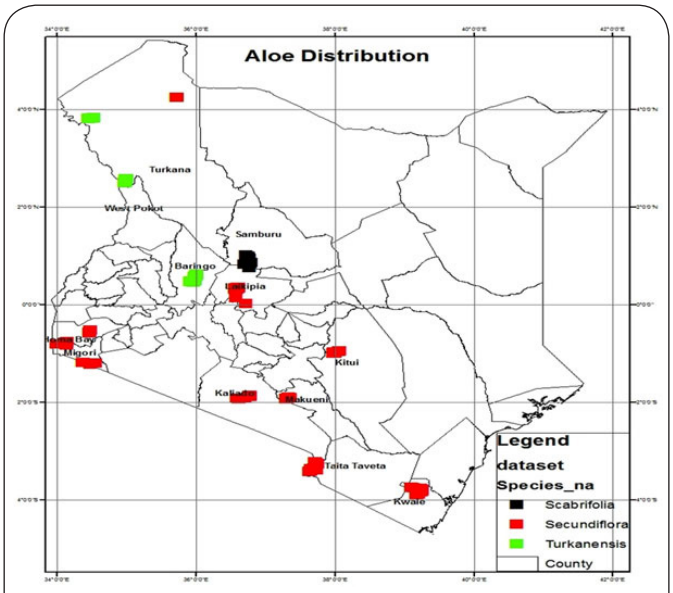

Figure 2. Geographical distribution of the three (3) *Aloe* species in the study areas in Kenya.

Aloe scabrifolia was found distributed only in the Northern part of Kenya in Laikipia and Samburu Counties. The species was found to inhabit bushlands dominated by *Acacia* spp, *Balanites aegyptica*, *Prosopis juliflora*, *Agave* spp and other *Aloe* spp under rocky loamy soils.

The genus *Aloe* is known mostly to be associated with dry habitats with few species colonizing the subtropics. The results about distribution from this study are in agreement with other findings [23,24]. However, *Aloe* species is reported to also thrive under closed-canopy forests at altitudes of 2700m absl [24]. The distribution of the three *Aloe* species studied seemed to follow a particular geographical range whereby *A. turkanensis* and *A. scabrifolia* were found distributed only to the North of the equator while *Aloe secundiflora* seemed to colonize both the North and South hemispheres [6,25] reported that the East Africa region alone had about 83 species of *Aloe* and approximately 60 species growing naturally in the dryland zones of Kenya. From this study, different *Aloe* species were found growing naturally in abundance in several Counties in Kenya including Baringo, Samburu, West Pokot, Turkana, Laikipia, Homabay, Migori, Kwale and Taita Taveta. [26] Found *Aloe* species growing naturally in Nyeri, Kiambu, Machakos, Kitui and western and coast regions of Kenya. [5], reported *Aloe* species habitat preferences in Kenya with 66% occurring in deciduous bushland/woodlands, 14% in grasslands while 20% for thickets, riverine woodlands and scrubland or rock outcrops. The same study revealed that the highest proportion of aloes (32%) occurred between 100-1500 m above sea level.

From other studies by [5], *A. secundiflora* occurs mostly in grassland and deciduous woodlands in both relatively wet to arid climatic zones and less in marshy or water-logged environments. Both *A. scabrifolia* and *A. turkanensis* are believed to be localized (special habitat), mostly found in harsher and more arid climates especially in northern Kenya, Southern Ethiopia and eastern Uganda, [5]. Such reports agree with the findings in this study whereby *A. turkanensis* and *A. scabrifolia* were found distributed only to the North of the equator (northern Kenya). In Kenya, population census for many specific *Aloe* species is lacking and hence difficulties in providing reliable estimates of population size important for conservation or even commercialization. Studies by [7] estimated population of commercial *Aloe species* to be 129 million plants, 83% of which were *A. secundiflora*. The other commercial species of interest like *A. turkanensis, A. calidophila* and *A. rivae* accounted for only 0.1% while the *A. scabrifolia* listed as endemic [5]

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was estimated at 16.9% of the total count. The geographical distribution of most plants is influenced mostly by both edaphic and climatic factors.

Since the soil type differs a lot in different ecological zones, perhaps this explains why the *A. turkanensis* and *A. scabrifolia* were found distributed in Northern Kenya as the soils are nearly similar in Turkana, Baringo and Samburu Counties compared to the coastal areas of Kenya. Therefore, it is recommended to undertake biophysical studies among the three commercial *Aloe* species to help in determining why the *A. secundiflora* was dominant when compared the other species.

Molecular characterization of the three commercial Kenyan *Aloe* species, using RAPD and ISSR markers species

In this study, all populations revealed clear polymorphism over the primers used as shown in **Table 1**. The Nei's genetic diversity indices for the combined markers (ISSR and RAPD) ranged from 0.112 to 0.177 in Baringo-TUR and Maralal-Sec populations respectively (**Table 2**). The percentage of polymorphic bands observed within the populations also ranged from 33.71% to 51.12%. The average gene diversity index among the population (G_{st}) was revealed to be 41% (**Table 2**) from both the RAPD and ISSR bands.

The highest genetic similarities were recorded between Kajiado and Gwasi, Laikipia-SEC and Isebania, and maralal-SEC and Kajiado populations at 0.996 while the lowest similarities were recorded between Laikipia-SCAB and Laikipia-SEC at 0.575. The hierarchical cluster analysis (UPGMA) categorized populations into three main groups (**Figure 3**). The first cluster A comprised of Baringo–TUR, Loima-TUR and Oropoi-TUR populations. The second cluster B consisted of Gwasi, Samburu, Isebania, Kajiado, Laikipia-SEC, Sultan, Lokitaung, Mwingi, Maralal SEC and Taveta populations. The last cluster C comprised of Laikipia SCAB and Maralal SCAB populations. The principal coordinate analysis (PCoA) performed on combined

Table 2. Population Genetic parameters: percentage of polymorphic band(s), Nei's genetic diversity (H), Nei's genetic differentiation index among populations (Gst).

	RAPD		ISSR		RAPD & ISSR	
	H	% of polymorphic bands	H	% of polymorphic bands	H	% of polymorphic bands
BARINGO TUR	0.0961 (0.1568)	37.38	0.1361 (0.1741)	52.11	0.1121 (0.1646)	43.26
GWASI	0.1398 (0.1863)	42.99	0.2044 (0.1929)	63.38	0.1656 (0.1911)	51.12
ISEBANIA	0.1231 (0.1782)	40.19	0.1817 (0.1915)	59.15	0.1465 (0.1853)	47.75
KAJIADO	0.1431 (0.1870)	42.99	0.2094 (0.1923)	63.38	0.1695 (0.1914)	51.12
LAIKIPIA SEC	0.1196 (0.1752)	39.25	0.1763 (0.1889)	57.75	0.1422 (0.1824)	46.63
LAIKIPIA SCAB	0.1279 (0.2013)	29.91	0.1201 (0.1673)	39.44	0.1247 (0.1880)	33.71
LOIMA TURK	0.1022 (0.1678)	33.64	0.1411 (0.1860)	45.07	0.1177 (0.1758)	38.2
LOKITAUNG	0.1293 (0.1816)	40.19	0.1898 (0.1926)	59.15	0.1534 (0.1879)	47.75
MARALAL SCAB	0.1310 (0.2055)	29.91	0.1433 (0.1937)	39.44	0.1359 (0.2004)	33.71
MARALAL SEC	0.1498 (0.1923)	42.99	0.2190 (0.1958)	63.38	0.1774 (0.1961)	51.12
MWINGI	0.1236 (0.1778)	40.19	0.1823 (0.1908)	59.15	0.1470 (0.1848)	47.75
OROPOI TUR	0.1124 (0.1711)	37.38	0.1567 (0.1886)	49.3	0.1301 (0.1791)	42.13
SAMBURU	0.1439 (0.1899)	42.99	0.2099 (0.1951)	63.38	0.1702 (0.1942)	51.12
SULTAN	0.1320 (0.1858)	40.19	0.1944 (0.1978)	59.15	0.1569 (0.1926)	47.75
TAVETA	0.1446 (0.1879)	42.99	0.2116 (0.1928)	63.38	0.1713 (0.1922)	51.12
Gene diversity among populations (Gst)	0.37		0.4		0.41	

H represents Nei's genetic diversity index within the population. Standard deviations are in ()

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binary data for RAPD and ISSR grouped the populations into three major clusters similar to the hierarchical cluster analysis (**Figure 4**).

Similarly, the analysis of molecular variance (AMOVA) calculated using Genalex 6.5 on the Nei's similarity matrices from the combined RAPD and ISSR primers, revealed significant variations (P>0.01) within and among populations at 58% and 42% respectively (**Table 3**).

Genetic diversity represents the heritable variation within and among populations of organisms. For effective plant breeding and germplasm collection, there is need to understand the extent of genetic diversity [27]. Molecular marker analysis is a reliable tool for assessment of genetic diversity among taxa but traditional methods like morphological traits have proved to be relatively less reliable and inefficient for discrimination of closely related genotypes in *Aloe* species [28]. Therefore, selection based genetic information using molecular markers is more reliable and consistent.

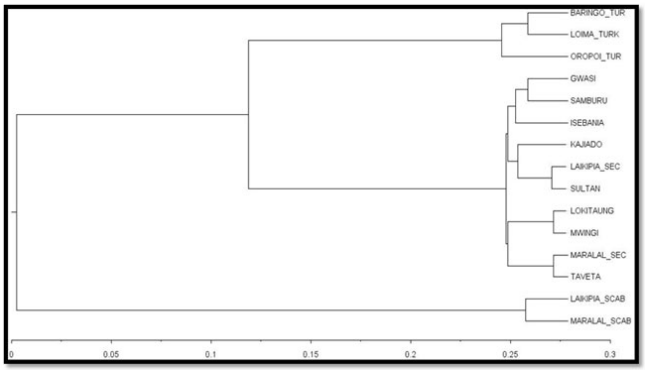

Figure 3. Dendrogram (UPGMA) representing genetic relationships among 15 populations of three indigenous *Aloe* species based on Nei's genetic similarity indices obtained using pooled combined RAPD and ISSR primers.

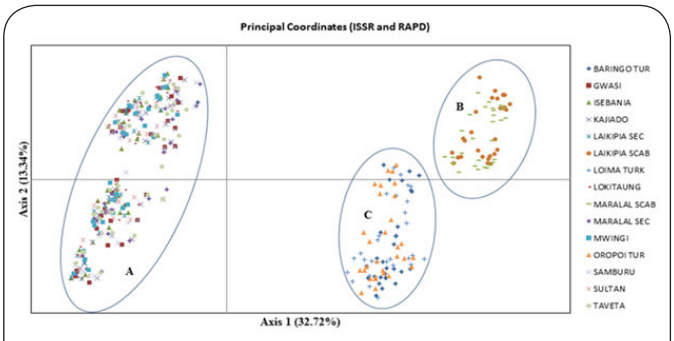

Figure 4. Two-dimensional scaling of Aloe populations by principal coordinate analysis using pooled data from combined RAPD and ISSR markers.

Table 3. Analysis of Molecular Variance (AMOVA).

Source of variation	df	SS	MS	Est. Var.	%	*P* (rand >= data)
Among Pops (ISSR)	14	2287.9	163.43	5.23	0.44	.001
Among Pops (RAPD)	14	2004.3	143.17	4.54	0.39	.001
Among pops (RAPD and ISSR)	14	4292.3	306.59	9.77	0.42	.001

This study also characterized fifteen *Aloe* populations using RAPD and ISSR markers to ascertain the genetic diversity within and among them for conservation, management and sustainable utilization of *Aloes*. The results revealed that all the loci detected by both RAPD and ISSR markers in *Aloe* species genotypes were polymorphic. A higher percentage of polymorphism was revealed by ISSR markers in comparison to RAPD markers (94% and 65% respectively). The percentage of polymorphic bands observed within the fifteen populations ranged from 29% to 42% in RAPD and 39% to 63% in ISSR markers. Similar findings have been reported in various studies; [29] obtained a higher polymorphism using ISSR markers (80.9 %) than RAPD (71.8 %) while studying genetic diversity of *Aloe vera*. However, low levels of polymorphism using both ISSR and RAPD markers were reported in *Bruguirra gymnorrhiza* and *Heritiera fomes* [30]. Most studies on wild plant populations have used the percentage of polymorphism as an indicator of genetic diversity [31]. However, [32] indicated that the percentage polymorphism is not a significant measure of genetic variation and that the parameter of genetic diversity (H) is more applicable.

In addition, there was also a higher variance in genetic diversity within populations than among populations when the RAPD, ISSR and when both markers were used. In this study, both ISSR and RAPD revealed significant variations among the *Aloe* populations yielding 44% and 39% respectively.

The combined ISSR and RAPD markers yielded 42% genetic variation among the fifteen populations. Since RAPD and ISSR markers have different strengths, it has been proposed that a combination of both markers in genetic studies is desirable for more accurate results. [33], reported the different abilities of ISSR and RAPD markers in discriminating different genotypes. The combination of markers might be a good approach due to the different regions targeted by each marker allowing for wide genome coverage in genetic variability studies. The ISSR markers have been reported to be highly reproducible and produce more complex banding patterns than RAPD [34-36]. In addition, the RAPD markers are known to cover the entire genome of coding and non-coding regions while ISSR markers reveal polymorphisms from sequences between two microsatellite primer sites [13,37]) and thus important when differentiating closely related cultivars or species. In the past, researchers have reported genetic diversity studies in *Aloe* species using RAPD and ISSR markers among other different marker systems. This is because of their simplicity, rapidness, affordability and can be applied without any prior knowledge regarding the plant genome [29,38]. Both RAPD and AFLP (Amplified Fragment Length Polymorphism) markers have also been used to study the genetic diversity among different *Aloe* species [39,40]. In

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India, [28,41] used RAPD and AFLP markers to assess genetic diversity among *Aloe* accessions from different geographical regions. Similar results have been reported by [10] and [29] on genetic variability in *Aloe vera* where both RAPD and ISSR primers have been used.

Moreover, in this study, there was genetic diversity in each population as revealed by the Nei's genetic diversity indices (H) which varied between the ISSR and RAPD markers. The RAPDs revealed genetic diversity ranging from 0.0961 (Baringo Tur) to 0.1498 (Maralal Sec), while ISSR markers ranged from 0.1201 (Laikipia Scab) to 0.219 (Maralal Sec). With the combined effects of the two markers, the genetic diversity indices within the populations ranged from 0.1121 (Baringo Tur) to 0.1774 (Maralal Sec). The higher genetic diversity recorded by the ISSR markers as compared to RAPD was attributed to its high power of discriminating between closely related genotypes [33]. The Nei's genetic diversity indices (H) also revealed high variation among individuals within a single population as observed with the average gene diversity among populations (Gst) being revealed at 0.37 and 0.4 for RAPD and ISSR markers respectively.

The genetic diversity results were in line with those of molecular variance analysis whereby, the variations among the populations were highly significant when both markers were used combined or separate. This therefore, indicated that the major factors endangering the survival of *Aloe* species in Kenya are both ecological and anthropogenic as supported by [42]. The pattern of genetic variations as portrayed by the genetic indices among the populations in this study might also be attributed to the cumulative genetic changes within each population and the difference in species. The results obtained could also be attributed to the pollination, propagation and seed dispersal mechanisms of *Aloe* species as they reproduce both vegetatively and by seed. This phenomenon combined with the long-lived perennial habit of the plants leads to predominance of older plants in *Aloe* populations [7,43].

In addition, the distribution of *Aloe* species is affected by the presence of specific pollinators and by seed morphology [44] whereby, some *Aloe* seeds such as those of *Aloe excelsa* have large, efficient wings that aid dispersal, and may account for their widespread distribution [44]. Other species such as *Aloe aculeata* produce wingless seeds, seemingly limiting their dispersal, thus resulting in dense stands of plants in localized areas [44]. Reduction and fragmentation in conserved forested landscapes and over-exploitation have been cited as the main causes leading to increased genetic differentiation and reduced gene flow between populations [45].

In conclusion, the ability to resolve genetic variation among different genotypes may be directly related to the number of polymorphisms detected with each marker technique. Therefore, studies on genetic variation in *Aloe* species should prioritize the combined use of both ISSR and RAPD markers as they reveal high variation when employed together. This will help in undertaking conservation measures especially *ex-situ* for those populations with economic importance like the Maralal SEC (*secundiflora*) population which had the highest genetic diversity.

Mapping of the genetic pools for the three commercial *Aloe* species (*A. secundiflora, A. turkanensis, and A. scabrifolia*) in ASALs of Kenya

In mapping the population genetic structure of the three *Aloe* species (*turkanensis, scabrifolia* and *secundiflora*), the genetic binary data for combined ISSR and RAPD markers were used to estimate the exact number of subpopulations on the basis of the maximum assumed or estimated populations (ΔK) values. Three groups were formed at K=3, which corresponded to the three *Aloe* species used in the study as shown in **Figure 5** and **Table 4**. The exact number of populations based on gene pool proximity was assessed by Structure Harvester software using admixture model assumptions with maximum ΔK value observed at K=3 (1569.3) as shown in **Table 4**. The *Aloe turkanensis* clustered in the first group (red), followed by *Aloe scabrifolia* (Green) and then *Aloe secundiflora* (Blue) as shown in **Figures 6-9** based on genetic structure and the hierarchical UPGMA analysis.

This study exhibited a population genetic structure with three major clusters which corresponded to the three *Aloe*

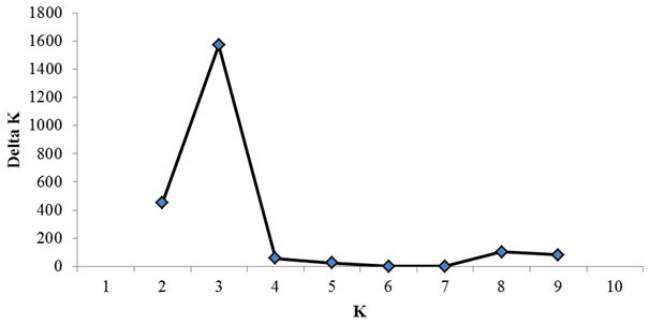

Figure 5. Estimation of populations (K) of the *Aloe* species genetic pools using Structure Harvester.

Table 4. Mapping of the *Aloe* species genetic pools using Structure Harvester.

K	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	-30756.8	0.353553	—	—	—
2	-18597.9	3.394113	2916.75	1537.55	453.005
3	-21514.7	4.030509	9242.1	6325.35	1569.368
4	-17218.7	5.515433	1379.2	322.9	58.54482
5	-16162.4	25.59727	1056.3	641.2	25.04955
6	-14464.9	1751.221	1697.5	1142.25	0.652259
7	-13909.7	1503.38	555.25	1163.6	0.773989
8	-12190.8	34.78965	1718.85	3555.1	102.1884
9	-14027.1	56.9221	-1836.25	4672.1	82.07885
10	-11191.2	115.5412	2835.85	—	—

doi: 10.7243/2050-2389-8-1

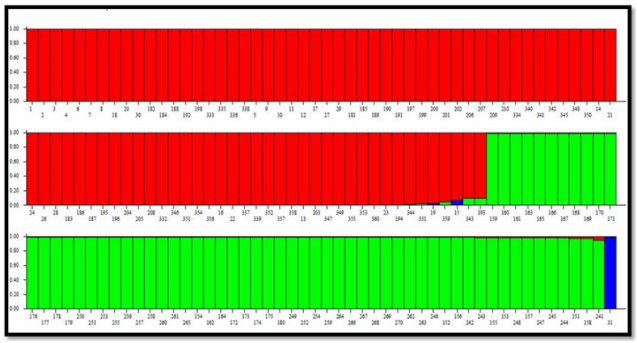

Figure 6. Mapping of the *Aloe* species genetic pools using Structure Harvester.

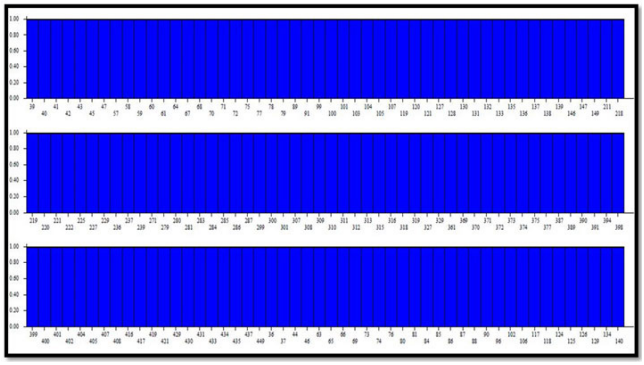

Figure 7. Mapping of the *Aloe* species genetic pools using Structure Harvester.

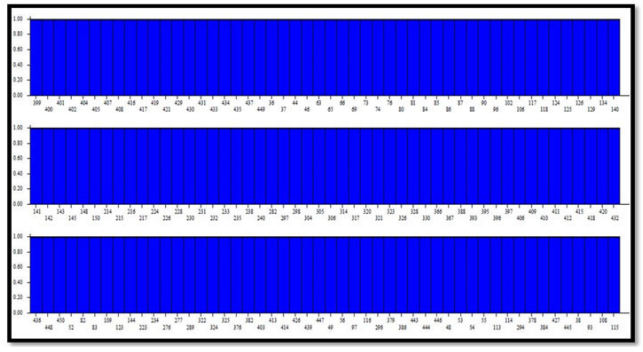

Figure 8. Mapping of the *Aloe* species genetic pools using Structure Harvester.

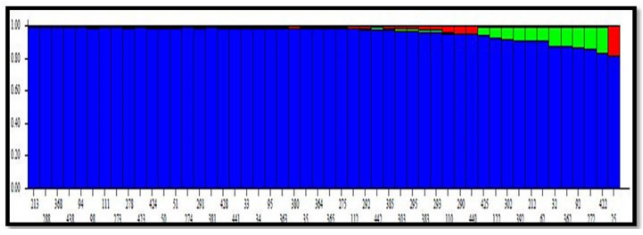

Figure 9. Mapping of the *Aloe* species genetic pools using Structure Harvester.

species (*turkanensis, scabrifolia and secundiflora*). The maximum assumed or estimated populations (ΔK) value was observed for K=3 corresponding to the three *Aloe* species studied. The overall membership proportion showed very minimal reproductive crossing between the *Aloe* species. The three clusters based on genetic structure and the hierarchical analysis for all the populations corresponded mostly with one another with respect to their pedigree relationships. However, the population genetic structure better explains the relationships because of the higher degree of simulation as explained by [21]; whereby, groupings based on larger value of ΔK could describe the number of subpopulations best fitted with the data rather than the higher LnP (K) value. The LnP(K) value estimates the posterior probability of the simulations which is then used to estimate the number of populations detected in the sample [46]. The genetic similarity analysis had also discriminated the *Aloe* populations into three broad clusters despite the high molecular variation among the fifteen populations. Both the principal coordinate and the UPGMA cluster analysis for each and the combined markers were in agreement from the results. These high variations could be attributed to, (i) the different *Aloe* species found in the different fifteen populations studied or (ii) the variations within each population as a result of genetic drift in a bid to adapt to new environment(s) through the loss of some alleles with successive generations in some populations.

According to [47], gene mapping is a process which allocates a relative position on a chromosome and the maps are species-specific comprised of gene markers and/or genes and the genetic distance between each marker. However, the genetic distances are said to be calculated based on the frequency of chromosome crossovers occurring during meiosis, and not on their physical location on the chromosome. The gene mapping or clustering a population structure is influenced by many factors including number of markers, sample sizes, the number of clusters and, allele frequency correlations etc. [48]. The mapping of the genetic structure may have also influenced the outcome of the genetic structure especially the model used either, Admixture or no Admixture models. In this study, Admixture model by the Structure Harvester Software was used to map the genetic data from the 15 populations. The Admixture model assumes individuals have an admixed ancestry whereby, each individual can inherit a fraction of their genome from ancestors in the population. However, [49] reported that the model ignores possible correlations in ancestry that occur in segments of each chromosome. In addition, the number of simulated clusters on the genetic data especially when employing the multidimensional scaling statistical methods influences the genetic structure [50].

Genetic diversity is simply the differences that occur among individuals of a species in the expression of a certain trait or set of traits and is crucial to population survival a given environment [51,52]. The interactions of individuals or populations may cause development of structures in species which is re-

vealed in the variability distributed within and among natural or artificial populations. For efficient conservation genetics management, it is important to involve population genetics models to save the threatened species from extinction [53]. Genetic variability is influenced by environmental factors such as edaphic, geographical and seasonal [54]. The adaptation of an organism to environmental changes requires a pool of variable genes in a population to withstand selective pressure [55]. The *Aloe* species are widely distributed in different habitats with varied edaphic and seasonal conditions [5] with their distribution indicating inherent genetic diversity that boosts adaptation to these conditions.

Mutations play a vital role in generation of new alleles in a population and this is geared by the evolutionary factors; natural selection, genetic drift and migration [53]. Thus, for success of any genetic conservation and breeding program, identification of the amount and distribution of genetic diversity in the gene pool of the concerned plant is important [10]. In conclusion, all these factors above may have resulted in the narrow and common gene pool in the three *Aloe* species populations. The differences in the results obtained may also be due to the fact that the two markers used target different portions of the genome [15,37].

Conclusions

From the results the following conclusions were made: *A. secundiflora* species was the most distributed in the studied ASALs of Kenya among the three *Aloe* species, being dominant in Coast, Lake and Central regions. There was high genetic variation among and within the studied populations. The genetic variation was highest within *A. secundiflora* species and least within *A. turkanensis* species. Maralal population had the highest genetic variation. The combined use of ISSR and RAPD markers revealed high genetic variation among the populations as compared to when either of them was used singly. The study exhibited a population genetic structure with three major clusters which corresponded to the three *Aloe* species (*turkanensis, scabrifolia and secundiflora*) with the overall membership proportion showing very minimal reproductive crossing between the *Aloe* species. It is therefore, recommended to undertake biophysical studies among the three *Aloe* species to help in determining why the *Aloe secundiflora* was dominant. In addition, *ex-situ* conservation for *Aloe turkanensis* should be undertaken as it had the least genetic variation and it was less distributed geographically. The Maralal *sec* population should also be conserved as it had high genetic diversity. Finally, studies on genetic variation in *Aloe* species should prioritize the combined use of both ISSR and RAPD markers as they reveal high variation when employed together.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	AA	GM	SN	JG	JK	JN
Research concept and design	✓	✓	--	--	✓	✓
Collection and/or assembly of data	✓	--	--	✓	--	--
Data analysis and interpretation	✓	--	✓	✓	--	--
Writing the article	✓	--	✓	--	--	--
Critical revision of the article	--	✓	✓	--	✓	✓
Final approval of article	✓	✓	✓	✓	✓	✓

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