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Assessing Genetic diversity of an endangered medicinal plant, *Strychnos henningsii* (Gilg.) in Nine Populations in Kenyan counties as revealed by ISSR Markers

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ABSTRACT

Strychnos henningsii (Gilg.) is an endangered medicinal plant in Kenya due to over exploitation for medicinal purposes. To understand the levels of genetic variation across populations and geographical regions of this species, we aimed at assessing the genetic diversity in nine Kenyan populations of Strychnos henningsii using inter-simple sequence repeat (ISSR) Markers. A total of 270 samples from nine populations of S. henningsii were collected. The genetic variation within and among populations were evaluated using inter-simple sequence repeat (ISSR). A total of 96 loci were revealed by ISSR primers all of which were polymorphic. These markers revealed that Ngong population was the most polymorphic with 51 (53.12%) polymorphic loci and Baringo as the least polymorphic with 28 (29.17%) polymorphic loci detected. Thirteen population specific loci were also revealed by

ISSR markers which might have contributed to specific population traits. A higher molecular variance was showed among populations 58% (p>0.001) than 41% within populations. According to Nei's unbiased genetic distance matrix the most genetically close populations were Taveta and Marsabit with the highest genetic identity (0.8803) and the lowest genetic distance (0.1275) between Jilore and Baringo. Clustering analysis based on Nei's genetic distance grouped the nine population into three; Cluster I consisted of three populations namely Kitui, Ngong and Jilore. Cluster II consisted of five populations (Marsabit, Taveta, Nyeri, Narok and Karura) and cluster III consisted of Baringo population. These results were also supported by principal coordinate analysis. The present findings indicated a low genetic variation between and among the existing genotypes of S. henningsii. This implies that the conservation efforts should aim to preserve all the existing populations of this threatened plant species along with extensive domestication for commercial purpose.

Key Words: *Strychnos henningsii, ISSR, genetic diversity, polymorphism, Conservation*

Introduction

Medicinal plants continue to be the most important source of drugs for the majority of world's population. Over the last decade there has been a rising demand for the herbal medicine globally (Panda *et al*., 2015; Varma and Shrivastava 2018) which is accompanied by dwindling supply of medicinal plants due to over-harvesting and habitat loss. Developing countries being the major suppliers of herbal materials are severely affected (Varma and Shrivastava 2018). Most of the harvested plant materials are derived from their natural habitats. This has led to a large number of medicinal plants under threat of extinction. Therefore there is a serious need for conservation and utilization of medicinal plants species. Studies on genetic diversity of medicinal plants can provide useful insights for designing conservation strategies and sustainable utilization of these genetic resources (Panda *et al*., 2015; Souza *et al*., 2012; Yang *et al*., 2012). As genetic diversity plays an important role in survival and adaptation of a species to its environment (Varma and Shrivastava 2018), it is essential to determine the amount of genetic variability using molecular markers.

Molecular markers are not influenced by environmental factors, are highly inheritable and exhibit enough polymorphism to discriminate against closely related genotypes (Archat *et al.,* 2003; Weising *et al*., 2005). There are several molecular markers available and which have been used in genetic diversity studies of medicinal plants. In the present study ISSR markers were used to determine the genetic variation among and within *S. henningsii* populations in Kenya. These markers have been used in genetic diversity studies of medicinal plants for example (Rocha *et al*., 2016) in *Croton heliotropiifolius*., (Brito *et al.*, 2016) in *Varronia curassavica* (Jacq.)., (Tabin *et al*., 2016) in Rheum, (Alansi *et al*., 2016) in *Ziziphus spina-christi* (L.) ,(Khan and Shah 2016) in *Withania somnifera* and (Pereira-Almeida *et al*., 2017) in *Croton tetrandenius.*

S. henningsii (Gilg.) is a plant species that belong to the family Loganiaceae and is widely distributed in the tropic and sub tropical regions. It has been used for a long time in the African traditional systems of medicinal practice for treatment of various disorders including rheumatism, gastrointestinal complications, abdominal pains, syphilis, and possibly of value in dysmenorrhoea (Hutchings 1989; Watt and Breyer 1962; Pujol 1993; Hutchings 1996; Oyedemi *et al.,* 2009). Because of its wide scale medicinal use, this plant species has been severely over-exploited in its natural habitats. In fact it has been reported as disappearing in Ukambani areas in Kenya (Musila *et al*., 2004). A research study on ethnobotany of *S. henningsii* (Kuria *et al.,* 2012) also indicated that this plant species was threatened in all the area of study. This has therefore called for conservation efforts for this valuable plants species to prevent from becoming extinct.

MATERIALS AND METHODS

Study areas and Plant materials

Nine populations were selected from areas where *S. henningsii* grows naturally. These populations were Taita-Taveta, Kilifi, Narok, Baringo, Kitui, Marsabit, Nyeri counties Karura forest (Kiambu county) and Ngong forest (Kajiado county) (Fig. 1). At least 30 individual plants were randomly selected for sampling in each population with about fifty to two hundred meters apart between them. Small discs of cambium tissues were collected directly from the tree trunk using a hollow metal and a hammer and preserved in absorbent envelopes containing silica gel until they arrived in the laboratory where they were dried in an oven at 30°c and the stored at room temperature until extraction (Esau 1977).

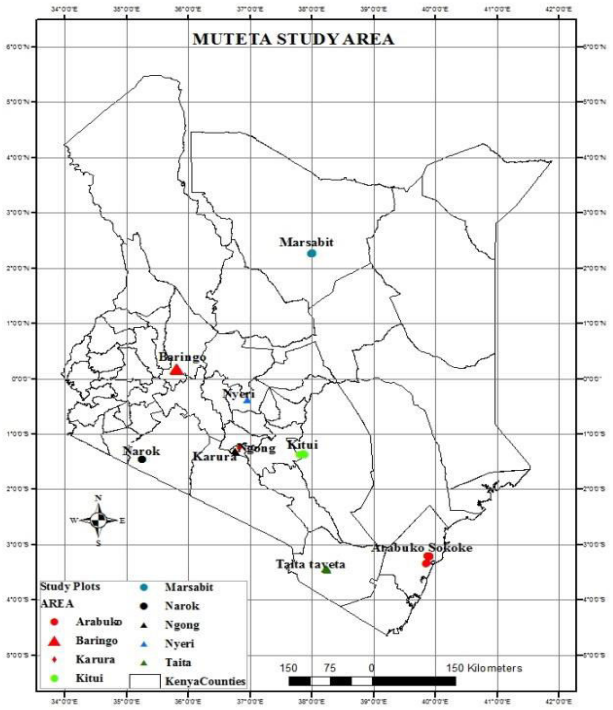

Fig 1: Map showing the sampling sites of S. henningsii in Kenya

DNA extraction

DNA was extracted from 0.1- 0.2g of cambium tissues using CTAB method by Doyle and Doyle (1990) method with modifications. A sorbitol buffer was initially utilized for removing mucilaginous polysaccharides, followed by tissue extraction with a CTAB buffer. To the powdered cambium tissues 500 µls of sorbitol buffer were added and mixed by vortex then centrifuged for 10 min at 14000 rpm at 4°c. The supernatant solution was removed and discard, then 750µl sorbitol buffer were added again and remix by vortex. The sorbitol buffer cleaning was repeated until no visible mucilage layer was present in the sample after centrifugation (usually 3 or 4 times). The supernatant solution was removed and 750µl CTAB buffer solution added and mixed well. The samples were incubated at 65°c in a water bath for 30-60 min and at RT with gentle inversions to mix. There after equal volume of (750µl) CIA was added and mixed by inversion for 10-20 min and then centrifuged at 14000 rpm at RT for 10-20 min. The upper aqueous solution was transferred into a new microfuge tube 2.0ml and equal volume (750µl) of

CIA added and mix by inversion for 10-20 min then centrifuged at 14000rpm at RT for 10-20 min. The upper aqueous solution was transferred into a new microfuge tube and 1/10 volume of 3M Ammonium acetate or Sodium acetate was added. Equal volume of isopropanol was added and the solution mixed by inversion then stored at -20°c overnight. The samples were centrifuged at 14000 rpm at 4°C for 5 min and 750µl of 70% ethanol was added to wash the DNA and this was repeated 2 to 3 times then centrifuged at 14000 rpm at 4°c for 5 min. The supernatant was discarded and the pellet dried and 100 µl TE added to dissolve the DNA. The DNA samples were subjected to the agarose gel electrophoresis and spectrophotometric analysis for assessment of their quality and quantity.

PCR-ISSR Assay

Forty ISSR (Invitrogen by Thermo Fisher Scientific) primers were screened and nine that gave clear and scorable bands were selected for their ability to amplify the genomic DNA of *S. henningsii.* The reaction mixture consisted of a total volume of 12.5µls which contained.12.5ng/µl DNA template, 1 Unit Taq polymerase, 10 × PCR buffer, 10mM dNTPS, 25mM $MgCl_2$, 10µM primers, 25% PVP and PCR grade water. The amplification was carried out in a 96 Veriti Applied Biosystems® thermacycler. This consisted of an initial 5 min denaturation step at 95^{o}C followed by 35 cycles of 30 s denaturation step at 95^{o}C, 45s annealing step at 47^{o}C, 2 min extension step at 72^{o}C and a final extension step for 5 min at 72^{o}C. The amplified DNA fragments for both RAPD and ISSR markers were separated by electrophoresis on a 2% agarose gel stained with 1 % SYBER SAFE dye in 1× TBE buffer at 100V for 3-4 h. DNA molecular marker (100-bp ladder) was also included in the gel to estimate the molecular weight of the DNA fragments obtained. After the run, the gels were photographed under UV light using gel documentation system and then scored for the presence/absence of a band for each primer .The data was recorded in MS excel for analysis.

Table 1: List of nine ISSR primers used for molecular characterization of S. henningsii

NO. ISSR.	Primer	Primer Sequence (5' -3')
1	UBC-807	AGA GAG AGA GAG AGA GT
2	UBC-809	AGA GAG AGA GAG AGA GG
3	UBC-811	GAG AGA GAG AGA GAG AC
4	UBC-818	CAC ACA CAC ACA CAC AG
5	UBC-820	GTG TGT GTG TGT GTG TC
6	UBC-825	ACA CAC ACA CAC ACA CT
7	UBC-830	TGT GTG TGT GTG TGT GG
8	UBC-861	ACC ACC ACC ACC ACC ACC
9	UBC-862	AGC AGC AGC AGC AGC AGC

Data Analysis

The binary data matrix in MS excel was subjected to POPGENE and GENEALEX software to determine the genetic diversity parameter for each population and for each marker. The percentage of polymorphic bands (PPB), total number of bands produced per primer, Nei's (1973) genetic diversity (H), Shannon information index (I) (Shannon and Weaver, 1949), Nei's (1978) unbiased genetic distance and Nei's genetic differentiation index (Gst) among population were estimated using POPGENE version 32 software (Yeh *et al*., 2000). Cluster analysis based on Nei's (1978) unbiased genetic distance was carried out using unweighted pair group method with arithmetic averaging (UPGMA) by Sneath and Sokal, 1973 using TFPGA Software (Miller, 1997) and a dendrogram was constructed using MEGA Ver. 7 (Tamura *et al*., 2016). Analysis of molecular variance (AMOVA) was carried out as described by Excofier *et al* (1992) using hierachial levels; individuals, populations and their groups to partition genotypic variance within and among *Strychnos henningsii* in Kenya. Analysis of molecular variance (AMOVA) and Principal Coordinate Analysis (PCA) were performed using GenAlEx 6.1 software (Peakall and Smouse, 2006).

RESULTS

270 individuals randomly selected from nine populations (Figure 1) of *S. henningsii* in Kenya were used for the genetic diversity study. Nine ISSR primers were selected which gave reproducible and distinct polymorphic amplified products (Plate 1).

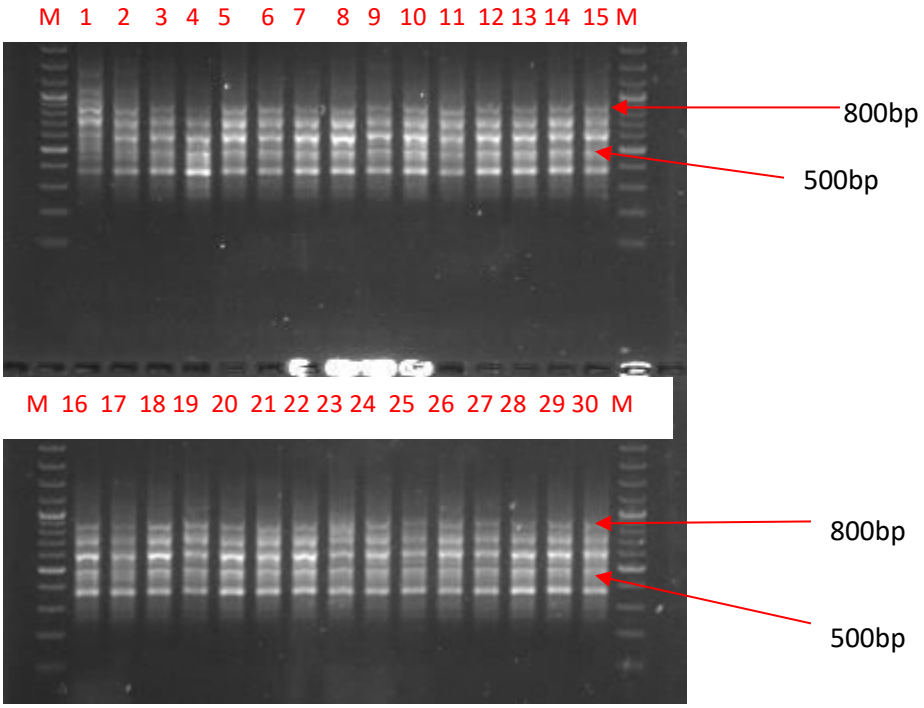

Plate 1: ISSR marker profile of amplified loci of samples from Baringo population using primer 862. Lane (1-30) are samples, M:-Marker DNA 100bp ladder

ISSR analysis revealed that Ngong population was the most polymorphic population with 51 (53.12 %) polymorphic loci detected while Baringo population was the least polymorphic population with 28 (29.17 %) polymorphic loci detected (Tables 2 & 3). The mean percentage polymorphism detected was 43.40 % (Table 2).

The nine primers revealed a total of 13 specific loci as follows: Kitui (5), Marsabit (1), Narok (2), Karura (2), Ngong (2), Taveta (2), Nyeri (0), Jilore (0) and Baringo (0) (Table 2). The number of observed alleles (Na) ranged from 1.2917-1.5312, Number of effective alleles (Ne) ranged from 1.1594 -1.3148, Nei's genetic diversity ranged from (H) 0.0955 - 0.1828 and Shannon information index (I) ranged from 0.1448-0.2728 (Table 3).

Table 2: Genetic diversity analysis of nine populations of S. henningsii estimated using ISSR markers in GenAlex software

Population	%P	N	Na	Ne	I	He	UHe	PSL
Kitui	43.75%	30.000	0.917	1.307	0.251	0.172	0.175	5.000
Marsabit	41.67%	30.000	0.865	1.255	0.219	0.147	0.149	1.000
Baringo	29.17%	30.000	0.688	1.159	0.145	0.096	0.097	0.000
Nyeri	39.58%	30.000	0.802	1.282	0.232	0.159	0.162	0.000
Narok	42.71%	30.000	0.885	1.271	0.230	0.156	0.158	2.000
Karura	51.04%	30.000	1.063	1.376	0.299	0.207	0.211	2.000
Ngong	53.13%	30.000	1.115	1.315	0.273	0.183	0.186	2.000
Jilore	37.50%	30.000	0.781	1.235	0.203	0.137	0.139	0.000
Taveta	52.08%	30.000	1.052	1.298	0.267	0.177	0.180	2.000
Mean	43.40%	30.000	0.907	1.278	0.236	0.159	0.162	

Table 3: Genetic diversity analysis of nine populations of S. henningsii estimated using ISSR markers in PopGene software

Population	N	PPL	%P	Na*	Ne*	H*	I*
Kitui	30	42	43.75	1.4375	1.3067	0.1720	0.2514
Marsabit	30	40	41.67	1.4167	1.2548	0.1469	0.2189
Baringo	30	28	29.17	1.2917	1.1594	0.0955	0.1448
Nyeri	30	38	39.58	1.3958	1.2823	0.1590	0.2317
Narok	30	41	42.71	1.4271	1.2715	0.1558	0.2303
Karura	30	49	51.04	1.5104	1.3764	0.2071	0.2994
Ngong	30	51	53.12	1.5312	1.3148	0.1828	0.2728
Jilore	30	36	37.5	1.3750	1.2346	1.1366	0.2030
Taveta	30	50	52.08	1.5208	1.2977	0.1773	0.2673
Overall	270	96	100	2.0000	1.4683	0.2889	0.4473

Key words:

N= population size, PPL= population polymorphic loci, % P= percentage polymorphism, Na = Number of observed alleles, Ne = number of effective alleles, H= Nei's genetic diversity, I = Shannon information indices, He = expected Heterozygosity, UHe = unbiased expected Heterozygosity, PSL= population specific loci

The polymorphism and expected heterozygosity revealed in this study were comparable to the results of the Principal Coordinate Analysis (PCA) where sample dispersion was highest in the more genetically diverse and the genetically similar populations. The first three axes accounted for 25.13 %, 19.78 % and 15.61 %; cumulative percentage being 60.52% (Figure 2) of the total genetic diversity detected in the entire study materials. According to Nei's (1978) unbiased measure of genetic identity and distance matrix (Tables 4), ISSR analysis revealed that the lowest genetic identity coefficient was 0.7239 (Jilore and Baringo populations) and the highest genetic identity coefficient was 0.8803 (Taveta and Marsabit populations) while the genetic distance ranged between 0.1275 (Taveta and Marsabit populations) and 0.3231 (Jilore and Baringo populations). Hence the most genetically diverse populations indicated from this analysis were Jilore and Baringo while Taveta and Marsabit were the most genetically close populations (Table 4). Analysis of molecular variance (AMOVA) revealed a higher genetic variation $p < 0.001$ (58 %,) among than within (41%) the *Strychnos henningsii* provenances (Table 5; Figure 3).

Table 3: ISSR population specific loci

population	NPL	primers	sequences	Band size (bp)
Kitui	5	807	AGA GAG AGA GAG AGA GT	400
		861	ACC ACC ACC ACC ACC ACC	900
		862	AGC AGC AGC AGC AGC AGC	500
		862	AGC AGC AGC AGC AGC AGC	400
		818	CAC ACA CAC ACA CAC AG	500
Marsabit	3	811	GAG AGA GAG AGA GAG AC	1000
		811	GAG AGA GAG AGA GAG AC	500
		862	AGC AGC AGC AGC AGC AGC	800
Karura	2	830	TGT GTG TGT GTG TGT GG	500
		861	ACC ACC ACC ACC ACC ACC	400
Ngong	2	825	ACA CAC ACA CAC ACA CT	300
		861	ACC ACC ACC ACC ACC ACC	500
Nyeri	0			
Jilore	0			
Baringo	0			
Narok	2	807	AGA GAG AGA GAG AGA GT	400
		809	AGA GAG AGA GAG AGA GG	400

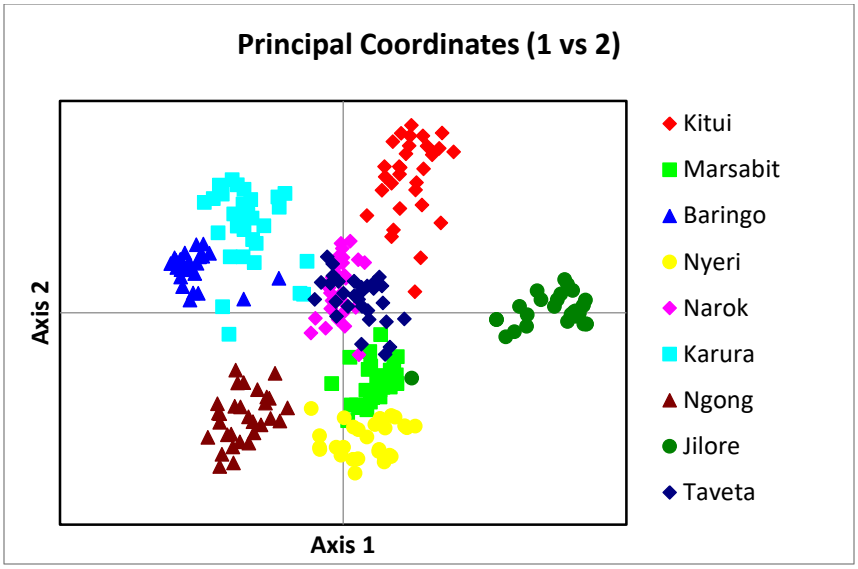

Fig 2: A three dimensional plot of the Principal Coordinate Analysis (PCA) of ISSR data showing the clustering of S. henningsii populations

***The first, second and third principal coordinates account for 25.13, 19.78% and 15.61% of total variation, respectively**

Table 4: Nei's (1978) Unbiased Measure of Genetic Identity and Genetic Distance from ISSR markers using PopGene software

pop ID	Kitui	Marsabit	Baringo	Nyeri	Narok	Karura	Ngong	Jilore	Taveta
Kitui	****	0.8556	0.7992	0.8141	0.8471	0.8422	0.8494	0.8428	0.8366
Marsabit	0.1560	****	0.8203	0.8755	0.8481	0.8417	0.8298	0.8337	**0.8803**
Baringo	0.2241	0.1981	****	0.8019	0.8361	0.8250	0.7675	**0.7239**	0.8004
Nyeri	0.2057	0.1330	0.2208	****	0.8606	0.8236	0.8064	0.8436	0.8639
Narok	0.1659	0.1648	0.1791	0.1502	****	0.8269	0.8146	0.8201	0.8705
Karura	0.1718	0.1723	0.1924	0.1941	0.1900	****	0.8074	0.7790	0.8713
Ngong	0.1632	0.1866	0.2647	0.2152	0.2050	0.2139	****	0.8363	0.8438
Jilore	0.1710	0.1819	**0.3231**	0.1700	0.1983	0.2497	0.1788	****	0.8314
Taveta	0.1784	**0.1275**	0.2226	0.1463	0.1387	0.1377	0.1698	0.1847	****

*Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 5: Analysis of Molecular Variance for S. henningsii as revealed by ISSR markers

Source	df	SS	MS	Est. Var.	%	p>0.05
Among Regions	2	669.272	334.636	0.269	2%	0.001
Among Pops	6	1868.083	311.347	10.139	**58%**	0.001
Within Pops	261	1876.100	7.188	7.188	**41%**	0.001
Total	269	4413.456		17.595	100%	

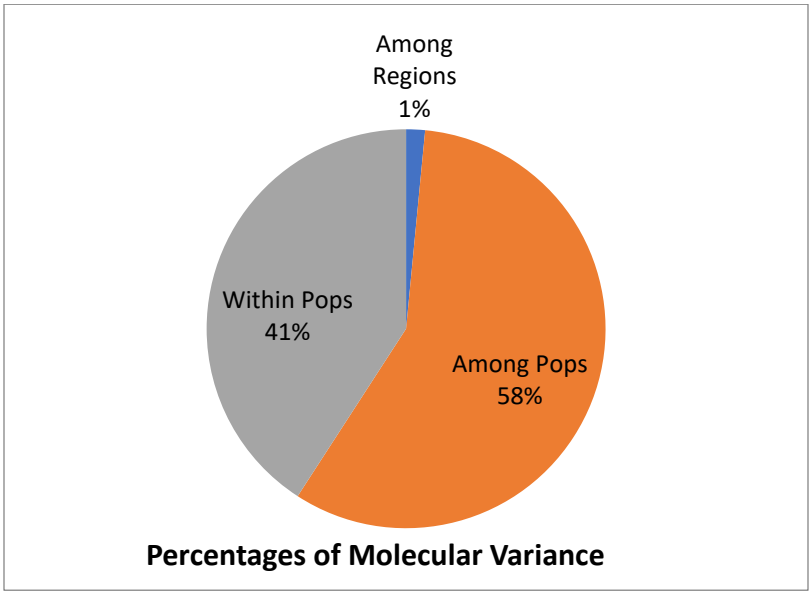

Figure 3: Percentage of Molecular variance of ISSR data

Cluster analysis of ISSR data based on the Nei's (1978) unbiased genetic distance generated a dendrogram with three major groups. Cluster I consisted of three populations namely Kitui, Ngong and Jilore. Cluster II consisted of five populations (Marsabit, Taveta, Nyeri, Narok and Karura) and cluster III consisted of Baringo population (Figure 4).

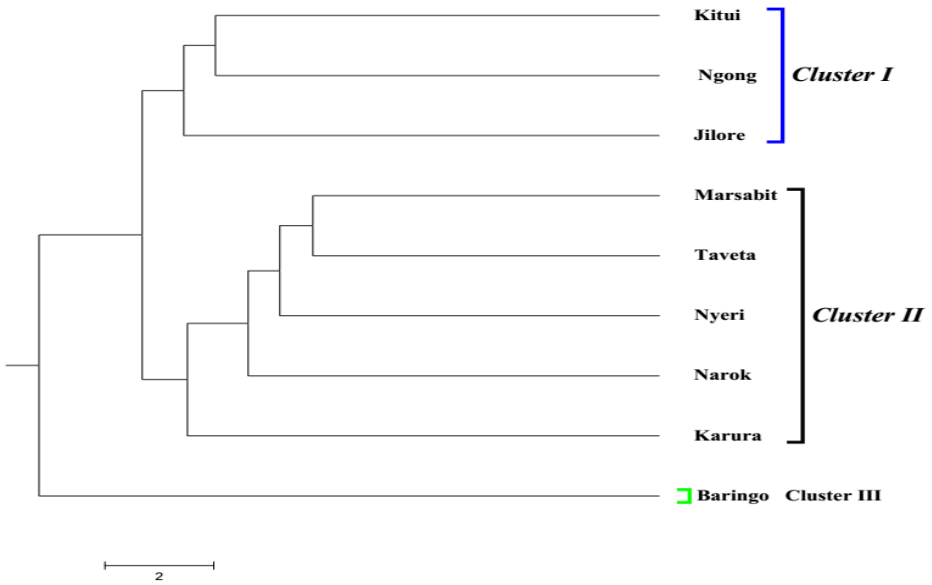

Figure 4: UPGMA clustering analysis of the nine S. henningsii populations based on Nei's (1978) unbiased genetic distance

DISCUSSIONS AND CONCLUSIONS

Genetic diversity assessment of medicinal plant species is an essential component in germplasm characterization, evolution, breeding and conservation (Wu *et al.,* 1999). However, from literature survey information on genetic diversity of *S. henningsii* is lacking. Therefore efforts were made in the present study to characterize 270 individuals from nine populations of *S. henningsii* in Kenya using ISSR markers in order to provide insight useful for its conservation and sustainable utilization. ISSR makers have been successfully used in other studies on genetic diversity of medicinal plant species such as (Rocha *et al*., 2016) in *Croton heliotropiifolius*., (Brito *et al.*, 2016) in *Varronia curassavica* (Jacq.)., (Tabin *et al*., 2016) in Rheum (Khan and Shah 2016) in *Withania somnifera* and (Pereira-Almeida *et al*., 2017) in *Croton tetrandenius.*

In the present study, ISSR markers detected and amplified 96 loci among *S. henningsii* genotypes all of which were polymorphic. The mean percentage polymorphism revealed by ISSR markers was 43.40%. The most polymorphic population revealed was Ngong with 51 polymorphic loci (53.12 %) while the least polymophic population was Baringo with 28 (29.17%) polymorphic loci. In other studies on the genetic diversity of *Costus pictus* similar results were obtained where a percentage polymorphism of 42.47% was revealed by ISSR markers (Naik *et al*., 2017). (Zebarjadi *et al.,* 2016) reported 59.13% percentage polymorphism *in Peganum harmals* L. Moreover, low levels of polymorphism in ISSR markers have also been reported in previous studies for example 24.36% in *Bruguirra gymnorrhiza* and 12.73% in *Heritiera fomes* (Dasgupta *et al.,* 2015). Yet in other studies a high polymorphism (94.8%) was reported in *Croton tetradenius* (Pereira –Almeida *et al*., 2017), *Ziziphus spina-christi* (L.) (Alansi *et al*., 2016) reported 93.4% percentage polymorphism and (Liu *et al*., 2013) reported 76.1% in *Thuja sutchuenensis.*

Several studies on natural populations have indicated the percentage polymorphism as a measure of genetic diversity (Naik *et al*., 2017). However, despite being the most commonly used indicator of genetic diversity, variation in this value is observed (Soares *et al*., 2016). Nei (1987) reported that percentage polymorphism is not a significant measure of genetic variation and that the parameter of genetic diversity (H) is more appropriate. In this

study, the values for genetic diversity (H) and Shannon index (I) ranged from (0.0955 - 0.1828) and (0.1448-0.2728). According to genetic diversity and Shannon index values, Ngong population was the most diverse and Baringo being the least diverse population. These values indicate a low genetic (allelic) diversity for *S. henningsii* genotypes. The results obtained could be attributed to the pollination, propagation and seed dispersal mechanisms in *S. henningsii*. This plant species has cleistogamous reproduction (self-pollinating) (Adekule 2015; Bruce and Lewis 1960) and bears small and brightly colored flowers which indicate a high possibility of entomophilous pollination. Insects transfer pollen for short distance mainly on flowers in a single tree resulting in the production of inbred seeds with poor germination (Adekule 2015; Bryndum and Hedgegart 1969; Mathew *et al*., 1987; Indira and Mohandas 2002; Tangmitcharoen *et al.,* 2009). It is propagated through seeds, wildings as well as suckers (Maundu and Tengas 2005). Seed dispersal is mainly by mammals especially birds and apes (Adekule 2015). It is also known to occupy restricted geographical zones in areas where it is naturally found. All these above factors have resulted in the narrow and common gene pool in *S. henningsii* populations. Other studies that reported low level of genetic divergence in the plant genotypes in their study include *Thuja sutchuenensis* (Liu *et al*., 2013), *Croton tetradenius* (Pereira –Almeida *et al*., 2017), *Costus pictus* Naik *et al*., 2017) and *Peganum harmals* L (Zebarjadi *et al.,* 2016).

Analysis of molecular variance (AMOVA) revealed a high (58%) genetic variation among populations than (41%) within populations. This may be due to the reproductive system in *S. henningsii*. Additionally, genetic drift may have also contributed to the higher among population diversity through the loss of some alleles with successive generations in some population. In *Withania somnifera* genetic diversity study, a high variation among populations than within populations was reported using RAPD and ISSR primers (Khan and Shah 2016). This was attributed to the self pollinating nature of the species. Panda *et al*., (2015) also reported a higher genetic variation among *Plumbago zeylanica* populations revealed by RAPD and ISSR markers due to habitat fragmentation. Reduction and fragmentation in wild medicinal plants due to over-exploitation in the forest cover could be one of the main causes that led to an increase in genetic differentiation and reduced gene flow between populations (Panda *et al.,* 2015). In this study, Kitui, Taita–Taveta and Nyeri populations were highly over exploited and thus revealed a high genetic variation due to the reduction in gene pool within these populations.

Clustering analysis grouped the populations into three groups based on the Nei's (1978) unbiased genetic distances and the Principal coordinate Analysis confirmed the results of the clustering analysis. However, both UPGMA and the PCA analysis did not indicate a clear pattern of clustering and the geographical trend among the populations. The genetic divergence did not match to the geographical places of collection. Similar results have also been reported in studies on other medicinal plants species such as (Zebarjadi *et al*., 2016) in *Peganum harmala* L. and (Liu *et al*., 2013) in *Thuja sutchuenensis* and (Pereira-Almeida *et al.,* 2017) in *Croton tetradenius* using ISSR markers while (Varma and Shrivasava 2018) in *Andrographis paniculata* used AFLP markers.

Geographical isolation is one of the major factor influencing genetic differentiation by limiting the amount of gene flow through seeds and pollen (Pfeifer and Jetschke, (2006). The lack of significant correlation between genetic distance and geographical locations indicate that genetic drift has played an important role in influencing the genetic structure and increasing the genetic variation among populations (Fischer *et al*., 2000). A decline in population size lowers genetic diversity and lead to inbreeding depression (Naik *et al*., 2015). This is a possible scenario in the analyzed populations of *S. henningsii*. This species was very common even within the vicinity of local people homesteads but has rapidly declined due to over exploitation in its natural habitats for medicinal purposes. This further accelerates the effects of genetic drift. In the long-term perspective, a reduction in genetic variation could lower the ability of a population to adapt and increase the risk if its extinction under changing environmental conditions (Liu *et al*., 2013).

In conclusion, the results of this study showed that low genetic diversity exist at species and population level as assessed by ISSR markers. However, high genetic variation among than within populations exists in *S. henningsii*. Therefore efforts should be made to preserve all the extant populations of this plant species and their habitat. Due to its wide scale medicinal use, it would be sustainable if plantation of new populations can be established to meet its demand. In this way we can alleviate the excessive collection of natural resources of *S. henningsii.*

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REFERENCES

Adebowale A (2015). Biosystematics studies in South African species of Strychnos L. Loganiaceae. A thesis desertification in University of Kwa Zulu- natal, Darban.

Alansi S, Tarroum M, Al-Qurainy F, Khan S, Nadeem M (2016). Use of ISSR markers to assess the genetic diversity in wild medicinal *Ziziphus spina-christi* (L.) Willd collected from different regions of Saudi Arabia, Biotechnology & Biotechnological Equipment, 30: (5): 942-947.

Archak S, Gaikward AB, Gautam D, Rao EVVB, Swamy KRM, Karihaloo JL (2003). Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale L.*) accessions of India. Genome. 46:362–369.

Brito FA, Nizio DAC, Silva AVC, Diniz LEC (2016). Genetic diversity analysis of *Varronia curassavica* (Jacq.) accessions using ISSR markers. Genetic Molecular Research 15: 1-10. http://dx.doi.org/10.4238/gmr.15038681

Bruce EA, Lewis J (1960). Loganiaceae. In: Hubbard CE, Milne-Redhead E. [eds.].Flora of Tropical East Africa. London.

Bryndum K, Hedgegart, T (1969). Pollination of teak (*Tectona grandis*). Silvae Gentica 18: 77-80

Dasgupta, N, Nandy P, Sengupta C, Da S (2015). RAPD and ISSR marker mediated genetic polymorphism of two mangroves *Bruguiera gymnorrhiza* and *Heritiera fomes* from Indian Sundarbans in relation to their sustainability. Physiology and Molecular Biology of Plants 21 (3): 375–384. doi: 10.1007/s12298-015-0308-0.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of Molecular Variance inferred from metric distances among DNA hyplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Fischer M, Husi R, Prati D, Peintinger M, Van Kleunen M, Schmid B (2000). RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). American Journal of Botany (87): 1128–1137.

Hutchings A (1989). A survey and analysis of traditional medicinal plants as used by the Zulu, Xhosa and Sotho. Bothalia 19: 111–123.

Hutchings A (1996). Zulu medicinal plants. Natural University Press. Pietermaritzburgs.

Indira EP, Mohandas K (2002). Intrinsic and extrinsic factors affecting pollination and fruit productivity in teak (*Tectona grandis Linn*. f.). Indian Journal of Genetics 63: 208-214.

Khan S, Shah PA (2016). Assessment of genetic diversity among India Ginseng, *Withania somnifera L.* Dunal. using RAPD and ISSR markers. Research Biotechnology 7: 01-10.

Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology. Evolution 33(7):1870–1874.

Kuria MW, Njenga P.K, Ngumi VW (2012). Ethnobotanical studies of *Strychnos henningsii* in five (Gilg.) natural habitats in Kenya. International Journal of Medicinal Plant Research 1 (6), 063-074, Available online at *www.internationalscholarsjournals.org*

Liu J, Shi S, Chang E, Yang W, Jiang Z (2013). Genetic Diversity of the Critically Endangered *Thuja sutchuenensis* Revealed by ISSR Markers and the Implications for Conservation. International Journal of Molecular Sciences 14: 14860-14871; doi:10.3390/ijms140714860

Mathew G, Mathew PK, Mohandas K (1987). Preliminary studies on insects visitors to teak (*Tectona grandis* L.f.) inflorescence in Kerala, India. Indian Forester 113: 61-64.

Maundu P, Tengäs T (2005). Useful Trees and Shrubs of Kenya. Technical handbook No. 35. Nairobi, Kenya: World Agroforestry center-Eastern and Central Africa regional Programme (ICRAF-ECA). Pp 400.

Miller MP (1997). Tools for Population Genetic Analysis (TFPGA), version 1.3. Flagstaff (AZ): Department of Biological Sciences, North Arizona University.

Musila W, Kisangau DP, Muema J (2004). Conservation status and use of medicinal plants by Traditional medicinal Practioners in Machakos district, Kenya; Traditional aspects of health and wellness. Proceedings of Inter- Institutional Consortium for indigenous knowledge, transforming the academy, Pennsylvania pg. 27-29.

Naik A, Prajapat P, Krishnamurthy R, Pathak JM (2017). Assessment of genetic diversity in *Costus pictus* accessions based on RAPD and ISSR markers. Biotechnology 7(1): 70-75.

Nei M (1987): Molecular Evolutionary genetics. New York, Columbia University Press. 522.

Nei M (1973). Analysis of gene diversity in subdivided populations. Genetics, 70: 3321-3323.

Nei M (1978). Estimation of average heterozygozity and genetic distance from a smaller number of individuals. Genetics, 89: 583-590.

Oyedemi SO, Bradley G, Afolayan AJ (2009). Ethnobotanical survey of medicinal plants used for the management of Diabetes mellitus in the Nkonkobe municipality of South Africa. Journal of medicinal plants research 3(12): 1040- 1044.

Panda,S, Naik D, Kamble A (2015). Population structure and genetic diversity of perennial medicinal shrubs Plambago. AoB PLANTS 7: plv048; doi:10.1093/aobpla/plv048.

Peakall R, Smouse PE (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology 6: 288-295.

Pereira -Almeida CS, Silvia AVC, Alves RP, Feitosa-Alcantara RB & Arrigoni-Blank ME (2017). Genetic diversity of mature populations of *Croton tetradenius* Baill. using ISSR markers. Genetic Molecular and Research 16 (2): 1-12.

Pfeifer M, Jetschke G (2006). Influence of geographical isolation on genetic diversity of *Himantoglossum hircinum* (Orchidaceae). Folia Geobotanica 41: 3–20.

Pujol J (1993). Naturafrica-the herbalists' handbook. Jean Pujol Natural Healer; Foundation 4Ed Durban Pergamum press 270.

Rocha TO, Freitas JS, Santos ESL,& Scaldaferri MM (2016). Estimate of genetic diversity in cassutinga (*Croton heliotropiifolius*) based on molecular markers. African Journal of Biotechnology 15: 518-523. http://dx.doi.org/10.5897/AJB2015.15009

Shannon CE, Weaver W (1949). The Mathematical Theory of Communication. University of Illinois Press, Urbana 290.

Sneath PHA, Sokal RR (1973). Numerical taxonomy. San Francisco (CA): WH Freeman.250

Soares ANR, Victória MF, Nascimento ALS & Ledo AS (2016). Genetic diversity in natural populations of Mangaba in Sergipe, the largest producer State in Brazil. Genetic Molecular Restoration 15: 1-12

Souza HAV, Muller LAC, Brandâo Lovato MB (2012). Isolation of high quality and polysaccharide free DNA from leaves of *Dimorphandra mollis* (Leguminous), a tree from the Brazilian Cerredo. Genetics and Molecular Research 11 (1): 756-764.

Tabin S, Kamili AN, Ganie SA & Zargar O (2016). Genetic diversity and population structure of *Rheum* species in Kashmir Himalaya based on ISSR markers. Flora 223: 121-128.

Tangmitcharoen S, Tasen W, Owen JN, Bhodthipuks J (2009). Fruit set as affected by pollinators of teak (*Tectona grandis L.*f.) at two tree spacing's in a seed orchard. Songklanakarin Journal of Science and Technology 31: 255-259.

Varma A, Shrivastava N (2018): Genetic structuring in wild populations of two important medicinal plant species as inferred from AFLP markers, *Plant Biosystems* - An International Journal dealing with all Aspects of Plant Biology, DOI:10.1080/11263504.2017.1418446.

Watt MP, Breyer NG (1962). Medicinal and poisonous plants of Southern and Eastern Africa. Livingstone, Edinburgh. 260

Weising K, Nybom H, Wolff K, Kahl G (2005). DNA fingerprinting in plants. Principles, methods and applications, 2Ed. Taylor & Francis Group, Bocaraton. 256

Wu J, Krutovskii KV, Strauss SH (1999). Nuclear DNA diversity, population differentiation and phylogenetic relationships in the Califonia closed-clone pines based on RAPD and allozyme markers. Genome 42:893-908.

Yeh F, Yang RC, Boyle T (2000). POPGENE—for the analysis of genetic variation among and within populations using co-dominant and dominant markers. Version 1.32.

Zebarjadi A, Ahmadvandi HR, Kahrizi D, Chenghamirza K (2016). Assessment of genetic diversity by application of inter simple sequence repeat primers on Iranian Harmal (*Peganum harmals* L.). germplasm as an important medicinal plant. Journal of Applied Biotechnology Reports 3 (3): 441-445.