

Genetic diversity and population structure of *Trichilia emetica* Vahl in western Kenya using ISSR markers

Elias Kiprotich Sirma

University of Eldoret

Benson Ouma Nyongesa

bnyongesa@uoeld.ac.ke

University of Eldoret

Otto George Dangasuk

University of Eldoret

Ken Patrick Andiego

University of Eldoret

Research Article

Keywords: Conservation genetics, Forest restoration, Genetic diversity, ISSR markers, Population structure, *Trichilia emetica*

Posted Date: November 3rd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7887580/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at New Forests on December 24th, 2025. See the published version at <https://doi.org/10.1007/s11056-025-10154-1>.

Abstract

Land-use changes can reduce genetic variation and limit the ability of tropical trees to adapt to climate change. *Trichilia emetica*, a versatile tree of ecological and economic importance in Kenya, remains uncharacterized at the genetic level. This study determined the genetic diversity and population structure of *T. emetica* in western Kenya using 15 Inter-Simple Sequence Repeat (ISSR) markers. A total of 171 DNA fragments were amplified, of which 94.65% were polymorphic, revealing substantial genomic variability. Overall, gene diversity was moderate ($H_e = 0.15$; $I = 0.22$), with Nandi (0.24) and Kakamega (0.18) populations showing the highest diversity while Kisumu and Siaya had the least (0.10). Analysis of molecular variance (AMOVA) partitioned 65% of total variation within populations and 35% among populations ($\Phi_{ST} = 0.35$, $p < 0.001$), indicating moderate differentiation and restricted but ongoing gene flow ($N_m = 2.39$). The six populations were clustered into three major genetic groups consistent with ecological gradients. This study establishes the first molecular baseline for *T. emetica* in Kenya and demonstrate that ecological heterogeneity may play a role in influencing its genetic structure. Conservation work should preserve the populations in Nandi and Kakamega while implementing enrichment and assisted regeneration in Siaya and Kisumu populations to reduce loss of genetic diversity.

Introduction

Most terrestrial biodiversity of the world is found in the forests that help provide ecological stability by regulating climate conditions, nutrients cycles, and supporting livelihoods. Nevertheless, uncontrolled, habitat fragmentation, logging, and unsustainable use of resources have caused extensive loss of genetic variation of trees, particularly in the tropics (Soares et al. 2019). Reduction in genetic diversity of intra-species undermines the adaptive ability of the population to environmental change and the resilience of forest ecosystems (Konrad et al. 2025). In such a way, the conservation of forest genetic resources has been on the global climate and biodiversity agendas, following the restoration objectives of the Bonn Challenge (International Union of Conservation of Nature [IUCN] 2011), Sustainable Development Goal 15 (Life on Land), and the United Nations Decade on Ecosystem Restoration (2021–2030), which put emphasis on the restoration and sustainable use of biodiversity to improve ecosystem resilience and human well-being (Forest and Trees, Agroforestry Flagship 1 2025; FAO 2025). Despite African forest ecosystems having ecological and socio-economic importance, the level of genetic research of native trees is underrepresented in comparison to Asia and Latin America. In recent genomic research, widely distributed species like *Azadirachta africana* and *A. quanzensis* are only beginning to be studied at the population level (Donkpegan et al. 2020). The lowland humidity forests and the arid woodlands, are fragmenting at a high pace being by other land uses. Such pressures interfere with gene flow and can result in or cause inbreeding, genetic diversity loss, and reduced evolutionary capacity, such as studies of *Acacia Senegal* where human activities predominantly define spatial genetic structure (Omondi et al. 2023). Thus, it is important to comprehend genetic diversity patterns and population structure in the context of evidence-based conservation, restoration, and sustainable management of African forest trees, as exemplified by the recent genetic analysis of *Vitellaria paradoxa* to inform West Africa

conservation planning (Attikora, et al. 2024). *Trichilia emetica* Vahl (Meliaceae), also known as Natal mahogany, is a multi-purpose tree species found widely in sub-Saharan Africa, from Senegal to the Red Sea, and moving eastwards and centrally through Africa to Congo and South Africa (PROTA 2025). It plays a significant ecological role in dry and moist forest ecosystems where it helps to establish the canopy structure, nutrient cycling, and habitat stability (Kenya Forestry Research Institute [KEFRI] 2021). It offers good-quality timber, which is valued to make furniture and construction, and its bark, seeds and leaves are also used in traditional ethnomedicine to treat fever, gastrointestinal disorders, and infection of the skins (Oyedeki-Amusa et al. 2021; Chebii et al. 2022). *T. emetica* also contains bioactive metabolites like limonoids, triterpenoids, and flavonoids, which have antimicrobial, antioxidant, and anti-inflammatory effects (Tsomele et al. 2021; Aldholmi et al. 2024). Furthermore, its seed oil possess good lipid profile with excellent oxidative stability, and could be a future nutraceutical and cosmetic ingredient source (Oyedeki-Amusa et al. 2021; Mabaso et al. 2025), and is proving to have an emerging economic payoff as a pharmaceutical and cosmetic industry feedstock. Morphological and phenological variation between the populations of *T. emetica*, such as leaf form, canopy architecture, fruit and seed size, and flowering time, has been reported in different ecological zones, which may be evidence of local adaptation and phenotypic plasticity (Akweni et al. 2021; KEFRI 2021; Tsomele et al. 2021). Although such variation indicates possible genetic differentiation, no population-level molecular analysis of *T. emetica* has yet been carried out to confirm it. Existing studies are restricted to morphological and ethnobotanical value, whereas molecular genetics has not been investigated in the populations of *T. emetica* in East Africa. In contrast, other genera of Meliaceae, e.g *Khaya* (Bouka et al. 2022), *Swietenia* (Alcalá et al. 2015; Limongi Andrade et al. 2022), *Cedrela* (Finch et al. 2022), *Azadirachta* and *Melia* (Cui et al. 2023), and *Toona* (Nie et al. 2025), have been analyzed using molecular and morphological markers, unveiling cryptic species boundaries, population differentiation, and adaptive divergence, of which were influenced by ecological and anthropogenic pressures. These findings demonstrate how habitat fragmentation, selective logging and ecological gradients can cause loss of genetic diversity and increase differentiation among populations. The absence of baseline molecular data for *T. emetica* is a critical knowledge gap in the evaluation of its intraspecific population structure and genetic diversity and limiting evidence-based conservation and restoration planning. With its ecological functions, commercial value, and extensive distribution, population structure and genetic diversity in *T. emetica* is required to form grounds for conservation and planning sustainable utilization.

Molecular markers give a precise means of revealing genetic diversity and population structure in forest trees, in particular, when the phenotypic variation is influenced by environmental factors. Co-dominant markers like simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs) offer high and accuracy resolution of alleles but are expensive to carry out and require relatively large amount of genomic resources (Alicandri et al. 2022; Faria et al. 2024). On the contrary, dominant multilocus markers like Inter Simple Sequence Repeat (ISSR) remain affordable and applicable to non-model and under-studied species (Viswanathan et al. 2018; Borah et al. 2021; Le and Le 2024). Specifically, ISSR prove to be invaluable since they are reproducible, do not need prior sequence information, and produce high polymorphic loci that enable one to make precise estimation of the genetic variation and

differentiation. They were shown to be able to identify population structure and gene flow in Meliaceae species, such as *Melia dubia* (Rawat et al. 2018). Even though ISSRs are not able to differentiate between homozygous and heterozygous loci, which is not advantageous as co-dominant markers, their high reproducibility and applicability in low-cost genotyping make them a reasonable genomic tool in genetic conservation and management (Alicandri et al. 2022). Without genomic resources in *T. emetica*, the ISSR markers will prove useful and helpful as a means to the end of creating a baseline level of knowledge about its genetic diversity and population structure.

Western Kenya is ecologically heterogenous with lowland, mid-altitude, and highland agroecological zones where rainfall, land-use, soil fertility differs (Jaetzold et al. 2006). The vast farming practices in region have led to land fragmentation which results in the disappearance of forests (Kogo et al. 2019; Rotich and Ojwang 2021; Osewe et al. 2022). Such landscape shifts may interfere with pollen and seed dispersal pathways, gene flow, population connectivity, and local tree species adaptation (Cheptou et al. 2017; Soares et al. 2019). *T. emetica* is a common in the ecological environments of natural forests and agroforestry in the region, which means its ecological strength and the intervention of mankind in in the spread of this tree species (KEFRI 2021). Such perseverance in diverse habitats where they exposed to anthropogenic activities is a challenge that can be exploited to unravel genetic traits of a species of conservation and socio-economicsignificance. As such, the current study aimed to determine genetic diversity and population structure of *T. emetica* in western Kenya using ISSR markers.

Materials and methods

Description of the study area

This study was carried out on six natural stands of *T. emetica* collected in Bungoma, Kakamega, Kisumu, Nandi, Siaya, and Vihiga in western Kenya, representing ecological and altitudinal variation in distribution of the species (Table 1). This region has varied agroecological zones, ranging from the lower midlands to upper highlands, with high variation in rainfall, temperature, and land use intensity (Jaetzold et al. 2006). The forest and woodland cover in western Kenya have been fragmented through agricultural land use and settlement (Kogo et al. 2019; Rotich and Ojwang 2021; Osewe et al. 2022), but *T. emetica* still occurs in both natural and modified environments. The locations were selected to capture such environmental diversity and to cover contrasting moisture and elevation, from the semi-arid Kisumu lowlands to the humid highlands of Nandi and Kakamega. Mean annual rainfall in the locations ranges from 1,100 mm in Bungoma to over 2,100 mm in Kakamega, while mean annual temperatures ranging from 20°C to 24°C (Jaetzold et al. 2006). Geographic coordinates, climatic conditions, and agroecological zones are presented in Table 1.

Table 1 Geographic and ecological characteristics of *T. emetica* populations sampled in western Kenya

Population	Altitude (m)	Latitude	Longitude	Temperature (°C)	Rainfall (mm)	Agroecological zones
Bungoma	1,700	0°34'10.29"N	34°33'30.15"E	20.3	1102	Lower highland
Kakamega	1,950	0°17'0.00"N	34°45'0.00"E	22.0	2100	Mid highland
Kisumu	1,131	0°5'30.12" S	34°46'4.64"E	23.0	1250	Lower midland
Siaya	1,525	0°3'45.46" N	34°17'16.11"E	23.4	2155	Lower midland
Vihiga	1,800	0°3'0.00" N	34°43'30.00"E	23.9	1900	Lower midland
Nandi	2,047	0°10'0.00" N	35°09'0.00"E	22.0	2000	Upper highland

Sampling design and leaf collection

A systematic random sampling approach was used in each population to minimize the chance of sampling genetically related individuals. Trees were sampled 50 m apart on 300–500 m transects traversing representative patches of habitat. Only healthy and mature trees with a diameter at breast height (DBH) ≥ 10 cm was sampled. For each tree sampled, fresh young leaves were in the middle-canopy were harvested and placed in sterile bags with silica gel and stored at -20°C before DNA extraction. Twenty individuals from each population were sampled in Bungoma, Kakamega, Kisumu, Siaya, Vihiga, and Nandi.

DNA extraction and purification

Genomic DNA was extracted from 0.5 g of leaf tissue by using the CTAB protocol optimized for phenolic-rich tree species (Doyle 1991). Leaf tissues were homogenized in liquid nitrogen and powder dispensed in 1.5% CTAB buffer (100 mM Tris-HCl pH 7.5; 1.4 M NaCl; 20 mM EDTA) supplemented with 0.75 μl β -mercaptoethanol and 100 mg polyvinylpyrrolidone (PVPP) to remove polyphenolic impurities. The homogenate was incubated at 60°C for 20 min, mixed with chloroform: isoamyl alcohol (24:1), and centrifuged at 13,000 rpm for 25 min. The aqueous phase was harvested, re-extracted with 10% CTAB, and precipitated with cold isopropanol. Nucleic acid was precipitated in ice-cold isopropanol and pellets washed in 70% ethanol, air-dried, and resuspended in 100 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). RNA was removed by treatment with RNase A (10 mg/mL) at 65°C for 3 h. Further purification was achieved by ethanol precipitation and re-suspension in 50 μl TE buffer. DNA quality and quantity were ascertained by electrophoresis on 1.5% agarose gels using unmethylated lambda (λ)DNA markers and spectrophotometry (A260/A280 ratio). DNA was diluted to approximately 30 ng/ μl for PCR amplification.

ISSR marker selection and PCR amplification

Fifteen **ISSR** primers (Table 2) previously optimized for other Meliaceae species like *Melia dubia* was used for polymorphism screening (Rawat et al. 2018). Those primers that gave reproducible clear

fragments duplicate reactions were selected for genetic diversity and population structure assessment. PCR amplifications were carried out in 25 µl reactions containing: 30 ng template DNA, 0.2 mM of each dNTP, 1.5 mM MgCl₂, 1× PCR buffer (with (NH₄)₂ SO₄), 2 U Taq DNA polymerase (Thermo Fisher Scientific), and 0.5 µM of each primer (Rawat et al., 2018). Amplifications were performed in an **Eppendorf Master cycler** (Germany) under the following conditions: initial denaturation at 94°C for 4 min; 39 cycles of 94°C for 30 s, primer-specific annealing (45–61°C) for 1 min, and extension at 72°C for 2 min; followed by a final extension at 72°C for 5 min. Amplicons were mixed with 1× bromophenol blue loading dye and electrophoresed on 2% agarose gels in 1× TAE buffer at 50 V for 2.5 h. DNA fragments were visualized under UV light after staining with ethidium bromide and gel image captured by a Herolab Gel Documentation System (Germany).

Table 2 ISSR primer sequences and amplification conditions used for PCR analysis of *T. emetica* populations in western Kenya

Marker code	Sequence (5'-3')	Tm (° C)	Ta (° C)
UBC -809	AGAGAGAGAGAGAGAGG	46.6	50.0
UBC -810	GAGAGAGAGAGAGAGAT	42.9	45.0
UBC -811	GAGAGAGAGAGAGAGAC	43.3	45.0
UBC -813	CTCTCTCTCTCTCTT	45.0	50.4
UBC -823	TCTCTCTCTCTCTCTCC	47.5	50.0
UBC -840	GAGAGAGAGAGAGAGAYT	45.8	47.0
UBC -845	CTCTCTCTCTCTCTCTRG	43.4	47.0
UBC -847	CACACACACACACACARC	54.2	53.0
UBC -855	ACACACACACACACACYT	60.2	61.0
UBC -857	ACACACACACACACACYG	57.1	58.0
UBC -864	ATGATGATGATGATGATG	51.2	52.0
UBC -880	GGAGAGGAGAGGAGA	49.0	44.7
UBC -888	BDBCACACACACACACA	52.3	55.4
UBC -890	VHVTGTGTGTGTGTGTG	51.8	52.0
UBC -891	VHVTGTGTGTGTGTGTG	51.8	55.0

Note: Tm = melting temperature, Ta = annealing temperature, B =C, G or T; R= A or G; Y = C or T; V = A, C or G and H = A, C or T.

Molecular data analysis

Only clear, well-resolved, and reproducible ISSR fragments were scored. Each distinct fragment was treated as a single locus and was scored manually for presence (1) or absence (0) in all individuals, following the binary data scoring approach of Rawat et al. (2018) and Borah et al. (2021). Consistently amplifying and polymorphic fragments were included in the final dataset. The resultant binary matrix was utilized for genetic diversity and population structure analyses. **Percentage of polymorphic loci (%P), number of effective alleles (Ne), Shannon's Information Index (I), and the coefficient of genetic differentiation (Gst)** were estimated using **PopGene version 1.32** (Yeh et al. 2000). These genetic indices provide complementary perspectives on within- and among-population genetic diversity and allow comparisons of allelic richness and differentiation (Nei 1973).

Analysis of Molecular Variance (AMOVA) was carried out using **GenAlEx version 6.5** (Peakall and Smouse 2012) to partition total genetic variation within and among populations. AMOVA was based on Φ -statistics, analogs of Wright's F-statistics, to quantify the amount of population differentiation and gene flow (Excoffier et al. 1992). Nei's unbiased genetic distance was used to compute pairwise genetic distances among individuals and populations (Nei, 1978). The **gene flow (Nm)** between populations was estimated indirectly from G_{ST} using the formula $Nm = 0.5 (1 - G_{ST}) / G_{ST}$ (Slatkin 1987). This index was used to infer the degree of genetic exchange and isolation among the populations of *T. emetica*. Genetic relationships among populations were estimated using a **Principal Coordinates Analysis (PCoA)** using **GenAlEx 6.5** (Peakall and Smouse 2012). A dendrogram was constructed based on Nei's genetic distance matrix using the **Unweighted Pair Group Method with Arithmetic Mean (UPGMA)** to infer relatedness among populations (Tamura et al. 2013). The robustness of cluster nodes was evaluated through **1,000 bootstrap replicates**.

Results

Genetic diversity within and among *T. emetica* populations

Fifteen ISSR primers produced a total of **171 scorable fragments, 162 (94.65%)** of which were polymorphic (Table 3). The number of amplicons per primer varied from **7 (UBC-857)** to **19 (UBC-810)**. Primers **UBC-809, UBC-845, UBC-847, UBC-857, UBC-864, UBC-880, and UBC-891** displayed 100% polymorphism. Polymorphism was 94.74%, 94.12%, and 93.75% for UBC-810, UBC-840, and UBC-888, respectively. On other hand, the lowest values of polymorphism were detected in **UBC-811 (84.62%), UBC-813 (87.50%), UBC-823 (87.50%), UBC-855 (90.00%), and UBC-890 (87.50%)**. The size of amplicons differed among primers and ranged from 169 bp in UBC-855 to 2,060 bp in UBC-880. A high fragment size range was observed with UBC-880 (178-2,060 bp), while UBC-809 produced the narrowest range of 315-389 bp.

Table 3 Polymorphism profile of 15 ISSR primers used for the genetic analysis of *T. emetica* populations in western Kenya

Marker code	Total fragments	Polymorphic fragments	Polymorphism (%)	Fragment size range (bp)
UBC -809	10	10	100.00	315-389
UBC -810	19	18	94.74	224-1420
UBC -811	13	11	84.62	183-1388
UBC -813	8	7	87.50	190-1265
UBC -823	8	7	87.50	213-1705
UBC -840	17	16	94.12	175-1390
UBC -845	8	8	100.00	470-1155
UBC -847	9	9	100.00	275-1367
UBC -855	10	9	90.00	169-1368
UBC -857	7	7	100.00	276-1895
UBC -864	12	12	100.00	290-1487
UBC -880	15	15	100.00	178-2060
UBC -888	16	15	93.75	201-1654
UBC -890	8	7	87.50	221-679
UBC -891	11	11	100.00	216-1672
Total	171	162	94.65	

Genetic diversity within populations

Table 4 shows the genetic diversity indices derived from ISSR marker analysis of six *T. emetica* populations in western Kenya. The **number of observed alleles (Na)** differed between populations from **0.66** in Siaya and Kisumu to **1.63** in Nandi with a mean of **1.02 ± 0.17**. Kakamega and Vihiga populations had intermediate Na values of 1.53 and 0.88, respectively, and Bungoma displayed a moderate value of 0.78. The **number of effective alleles (Ne)** ranged from **1.17** in Siaya to **1.40** in Nandi, with a mean of **1.24 ± 0.06**. Kakamega and Bungoma populations registered intermediate Ne values of 1.29 and 1.25, respectively, while Kisumu (1.18) and Vihiga (1.17) had lowest values. **Shannon Information Index (I)** displayed similar pattern of variation among populations, ranging from **0.15** in Siaya to **0.36** in Nandi, with a mean value of **0.22 ± 0.05**. Intermediate index values of 0.29 and 0.21, were observed in Kakamega and Bungoma, respectively, while lowest values were found in Kisumu (0.16) and Vihiga (0.16). The **Nei's gene diversity (He)** also differed among populations with a mean of **0.15 ± 0.03**. The **lowest He values were found in Siaya and Vihiga at 0.10, followed by Kisumu (0.11) and Bungoma (0.14), which registered relatively high gene diversity. Kakamega (0.18) was moderate for the He value, while Nandi (0.24) had the highest gene diversity among all the populations.**

The **percentage of polymorphic loci (%P)** varied among the six *T. emetica* populations. It ranged from **31.25% in both** Siaya and Kisumu populations to **78.31%** in Nandi, a mean of **48.96% ± 8.94**. Kakamega population had a relatively higher I percentage of polymorphism (75.00%) like that that in Nandi (78.31%), but lower percentage of polymorphism were observed in Siaya and Kisumu at 31.25%. Bungoma and Vihiga populations had 34.38 and 43.75%, respectively.

Table 4 Genetic diversity parameters based on ISSR markers across six *T. emetica* populations in western Kenya

Population	Na	Ne	I	He	%P
Bungoma	0.78 ± 0.17	1.25 ± 0.07	0.21 ± 0.05	0.14 ± 0.04	34.38
Kakamega	1.53 ± 0.15	1.29 ± 0.06	0.29 ± 0.04	0.18 ± 0.03	75.00
Kisumu	0.66 ± 0.17	1.18 ± 0.06	0.16 ± 0.05	0.11 ± 0.03	31.25
Siaya	0.66 ± 0.17	1.17 ± 0.07	0.15 ± 0.04	0.10 ± 0.03	31.25
Vihiga	0.88 ± 0.18	1.17 ± 0.06	0.16 ± 0.04	0.10 ± 0.03	43.75
Nandi	1.63 ± 0.13	1.40 ± 0.07	0.36 ± 0.05	0.24 ± 0.04	78.31
Mean ± SE	1.02 ± 0.17	1.24 ± 0.06	0.22 ± 0.05	0.15 ± 0.03	48.96 ± 8.94

Note: *Na* = number of observed alleles; *Ne* = number of effective alleles; *I* = Shannon's Information Index; *He* = Nei's gene diversity; *%P* = percentage of polymorphic loci.

Molecular genetic differentiation

Sixty five percent of total genetic variation was found **within populations and e 35%** was observed **among populations** (Table 5). The **fixation index (ΦST)** was estimated to be **0.35**, confirming that approximately 35% of the total genetic diversity was attributed to differences between populations.

Table 5 Analysis of molecular variance showing partitioning of genetic variation among and within six *T. emetica* populations in western Kenya based on ISSR marker data

Source of variation	Df	SS	MS	Estimated variance	Percentage of total variation (%)	ΦST	p-value
Among populations	5	140.72	28.143	1.29	35	0.35	<0.001
Within populations	114	272.40	2.389	2.39	65	—	—

Note: df = degrees of freedom; SS = sum of squares; MS = mean square; ΦST = fixation index representing the proportion of total genetic variation among populations. The ΦST value was calculated

as $1.288 \div 3.677 = 0.35$. Significance of variance components was tested using 999 random permutations ($p < 0.001$).

The overall **genetic differentiation coefficient (Gst)** of all loci was **0.27** ($p = 0.001$), as analyzed through 15 ISSR loci (Table 6). Uneven genetic differentiation was observed in each locus, with **Gst** ranging from **0.04 (UBC-811)** to **0.46 (UBC-888)**. Intermediate Gst values were found for some primers like **UBC-809 (0.35)**, **UBC-810 (0.31)**, **UBC-845 (0.35)**, **UBC-864 (0.34)**, and **UBC-891 (0.36)**. Lower differentiation estimates were obtained for **UBC-847 (0.08)**, **UBC-855 (0.05)**, **UBC-857 (0.16)**, and **UBC-880 (0.08)**. The corresponding **total genetic diversity (Ht)** among loci ranged from **0.02 (UBC-811)** to **0.50 (UBC-864)**, while different primers such as **UBC-810 (0.49)**, **UBC-840 (0.50)**, **UBC-888 (0.50)**, **UBC-890 (0.50)**, and **UBC-891 (0.49)** had relatively high total heterozygosity. The **within-population genetic diversity (Hs)** values ranged from **0.02 (UBC-811)** to **0.36 (UBC-840)**, averaging **0.24** across loci. **Gene flow estimates (Nm)**, whose relationship is inversely correlated with Gst, were variable between loci, ranging from **0.59 (UBC-888)** to **11.40 (UBC-811)** and averaging 2.39. Loci like **UBC-847 (Nm = 5.60)**, **UBC-855 (Nm = 9.12)**, and **UBC-880 (Nm = 5.67)** displayed relatively higher Nm values, while **UBC-809 (Nm = 0.93)**, **UBC-845 (Nm = 0.93)**, **UBC-864 (Nm = 0.96)**, and **UBC-890 (Nm = 0.64)** had lower estimates.

Table 6 Coefficients of genetic differentiation across 15 ISSR loci in six *T. emetica* populations from western Kenya

Marker code	Ht	Hs	Gst	Nm*
UBC-809	0.29	0.19	0.35	0.93
UBC-810	0.49	0.34	0.31	1.13
UBC-811	0.02	0.02	0.04	11.40
UBC-813	0.24	0.14	0.41	0.71
UBC-823	0.22	0.16	0.29	1.24
UBC-840	0.50	0.36	0.29	1.25
UBC-845	0.45	0.29	0.35	0.93
UBC-847	0.28	0.26	0.08	5.60
UBC-855	0.28	0.26	0.05	9.12
UBC-857	0.15	0.13	0.16	2.63
UBC-864	0.50	0.33	0.34	0.96
UBC-880	0.14	0.13	0.08	5.67
UBC-888	0.50	0.27	0.46	0.59
UBC-890	0.50	0.28	0.44	0.64
UBC-891	0.49	0.31	0.36	0.89
Mean	0.34	0.21	0.27	2.39

Note: *Ht* = total genetic diversity; *Hs* = within-population genetic diversity; *Gst* = coefficient of genetic differentiation; *Nm* = estimate of gene flow, calculated as $Nm = 0.5(1 - Gst) / Gst$ $Nm = 0.5(1 - Gst) / Gst$.

Genetic relationships among populations

A clear spatial distribution of genetic relationships among the six *T. emetica* populations in western Kenya (Fig 1). The first two principal coordinates accounted for the maximum percentage of overall genetic variance and produced a two-dimensional arrangement of genetic relationships among populations. The biplot showed distinct clustering with respect to population origin, and each grouping displayed differences in the level of dispersion. The **Nandi** population were plotted separately from the other populations, forming a distinct cluster along the first coordinate axis. The **Vihiga** population was positioned near the Nandi cluster but showed partial overlap with it and hence intermediate placement in the coordinate space. **Siaya** and **Kisumu** populations clustered in proximity to one another, while **Bungoma** and **Kakamega** formed a separate distinct cluster on the biplot.

Pairwise **Nei's genetic distances** among the six populations of *T. emetica* varied from **0.02** between **Vihiga and Kisumu** to **0.24** between **Bungoma and Nandi**, while the **genetic identities** differed from **0.79** to **0.98** (Table 7). Low genetic distances were reported for several population pairs, such as **Kisumu–Siaya (0.031)**, **Siaya–Vihiga (0.03)**, and **Kisumu–Vihiga (0.02)**, all of which also showed high genetic identity values exceeding **0.97**. Moderate distances were observed for **Kakamega–Siaya (0.09)**, **Kakamega–Vihiga (0.10)**, and **Bungoma–Kakamega (0.03)**, with identity values of **0.91**, **0.90**, and **0.97**, respectively. Larger genetic distances were found in the pairs that involved the Nandi population, especially **Bungoma–Nandi (0.24)**, **Siaya–Nandi (0.15)**, **Vihiga–Nandi (0.15)**, and **Kisumu–Nandi (0.13)**, and these had relatively low genetic identities of **0.79** and **0.88**, respectively. Comparison involving **Bungoma–Siaya (0.16)** and **Bungoma–Vihiga (0.18)** also involved relatively large distances.

Table 7 Pairwise Nei's genetic identity and genetic distance among six *T. emetica* populations from western Kenya based on ISSR marker data

Population	Bungoma	Kakamega	Kisumu	Siaya	Vihiga	Nandi
Bungoma	****	0.9742	0.8793	0.8487	0.8330	0.7867
Kakamega	0.0262	****	0.9417	0.9097	0.9015	0.8734
Kisumu	0.1286	0.0601	****	0.9714	0.9767	0.8759
Siaya	0.1640	0.0946	0.0291	****	0.9744	0.8612
Vihiga	0.1827	0.1037	0.0236	0.0259	****	0.8612
Nandi	0.2399	0.1354	0.1325	0.1494	0.1494	****

Note: Values above the diagonal represent Nei's genetic identity; values below the diagonal represent Nei's genetic distance.

Fig 2 shows the genetic relationships among the six populations of *T. emetica* as defined by the **Nei's unbiased genetic distances**. The dendrogram produced three clusters, indicating discrete levels of genetic relatedness among *T. emetica* populations. Siaya, Vihiga, and Kisumu were grouped in the same cluster and showed minimum separation in branch length within the cluster. The second cluster consisted of **Bungoma** and **Kakamega**, a closely related group different from Siaya-Vihiga-Kisumu cluster. On other hand, Nandi population was grouped in the third cluster, demonstrating its discreteness from the rest of the populations.

Discussion

Genetic diversity of *T. emetica* populations

The populations of *T. emetica* from western Kenya showed high mean percentage of polymorphic loci (94.65%), demonstrating discriminative power of ISSR markers to detect genetic variation. The high level

of polymorphism observed in this study is line with 91.7% reported in *Melia dubia* (Rawat et al. 2018), 95–97% in *Pinus sylvestris* (Sheikina and Romanov 2024), 80–100% in *Osyris lanceolata* (Mugula et al. 2023) and higher than 63.8% and 46–76% in *Parashorea chinensis* and *Robinia pseudoacacia* (Li et al. 2024; Uras et al. 2024), respectively, which shows the potential of ISSR in dissecting genetic diversity in tree species. The 100% polymorphism observed in several ISSR primers such as UBC-809, UBC-845, UBC-847, UBC-857, UBC-864, UBC-880, and UBC-891 indicates that ISSR tool is highly effective in detecting genetic variation in the *T. emetica* populations. Borah et al. (2021) also reported similar reliability of ISSR markers in showing high levels of genetic polymorphism in *Illicium griffithii*, further confirming their effectiveness in analyzing intra-specific variation in tree species.

The fragment sizes of amplicons ranged from 169 to 2060 bp further reinforcing the suitability of ISSR markers for assessment of genetic diversity in tree species and demonstrates wide genome variability among the *T. emetica* populations. Such high variation in fragment size shows the presence of several polymorphic loci distributed across the genome of *T. emetica* populations, indicating high allelic diversity, in line with its wide ecological distribution over lowland, mid-altitude, and highland habitats (Chebii et al. 2022; Osewe et al. 2022). Similar findings have been reported in *Khaya anthotheca* (Bouka et al. 2022) and *Cedrela odorata* (Finch et al. 2022), where wide ecological distribution was associated with high intra-specific polymorphism, indicating that tropical tree species with extensive habitats have large effective population sizes and diverse genetic backgrounds. The variation between primers in the percentage of polymorphism and the sizes of amplicons may indicate possible underlying differences in genome structure or localized selection pressures operating throughout *T. emetica* populations. These results are in line with recent findings in other forest trees such as *Acacia Senegal*, *Parashorea chinensis*, *Juniperus* spp., and *Anadenanthera colubrina*, where variation among ISSR primers in the percentage of polymorphism and fragment sizes was attributed to differences in genome structure and localized environmental influences (Omondi et al. 2023a; Xu et al. 2024; Al-Yasi and Al-Qthanin 2024).

The diversity indices in this study show the existence of genetic heterogeneity among the populations of *T. emetica* in western Kenya. Nandi population displayed the highest diversity indices ($N_a = 1.63$; $N_e = 1.40$; $H_e = 0.24$; $I = 0.36$; $\%P = 78.31\%$), indicating substantial allelic richness, evenness, and heterozygosity. These indices demonstrate that the Nandi population may be maintaining a large effective size and experiences high gene flow, with reduced human activities, which allow preservation of higher genetic variability. These findings are line with the previous ISSR and SSR-studies showing relatively high within-population diversity attributed to large, continuous populations, extensive gene flow, and open pollination systems that facilitate allelic exchange and reduced genetic drift in Meliaceae trees species such as *Melia dubia* (Rawat et al., 2018), *Swietenia macrophylla* (Limongi Andrade et al. 2022) and *Khaya anthotheca* (Bouka et al. 2022). Congruent with the results in this study, populations of *Memecylon subcordatum* (Viswanathan et al. 2018), *Osyris lanceolata* (Mugula et al. 2023), *Pinus sylvestris* (Sheikina and Romanov 2024) also had both high Shannon's diversity indices and percentage of polymorphic loci, demonstrating the influence of population size and ecological integrity in determining within-population genetic variation.

In contrast, Siaya and Kisumu *T. emetica* populations showed a lower diversity index ($N_a \approx 0.66$; $H_e \approx 0.10$; $\%P = 31.25\%$), indicating reduced allelic variation and possible genetic erosion. The reduced allelic richness and heterozygosity in Siaya and Kisumu correspond with documented pressures of deforestation, agricultural expansion, and unsustainable resource extraction in western Kenya (Kogo et al. 2019; Rotich and Ojwang 2021; Chebii et al. 2022; Osewe et al. 2022). Similar trend where habitat fragmentation and land-use change contributed to reduction in genetic diversity and gene flow in tropical tress species (Soares et al. 2019). A reduced gene diversity in isolated or heavily exploited populations has also been reported in *Melia dubia* (Rawat et al. 2018), *Khaya anthotheca* (Bouka et al. 202) and *Swietenia macrophylla* (Limongi Andrade et al. 2022). Bungoma, Kakamega, and Vihiga populations had moderate diversity indices ($H_e = 0.14-0.19$; $I = 0.21-0.29$; $\%P = 43-75\%$), indicating partial preservation of genetic diversity, reflecting their position along a continuum of anthropogenic distance and ecological connectivity. Therefore, the Bungoma-Kakamega-Vihiga populations may still be exchanging genes through fragmented corridors or remnant trees in agricultural landscapes, a pattern observed in *Tabebuia rosea* (Ruiz-González et al. 2023) and *Acacia senegal* (Omondi et al. 2023), where exchange of genes among isolated or remnant populations has been maintained despite fragmentation of the habitats.

Population differentiation and structure of *T. emetica*

The populations of *T. emetica* in western Kenya showed a structured yet genetically diverse pattern, influenced by limited gene flow. Sixty-five percent of the total genetic variation was observed within populations and 35% among the populations ($\Phi_{ST} = 0.35$), showing moderate to high genetic differentiation. The G_{ST} value (0.27, $p = 0.001$) reinforced this pattern, indicating that while diversity of alleles is preserved within the populations, a significant percentage of variation is spread among them. In comparison with other Meliaceae species, *T. emetica* displayed higher population differentiation than *Khaya anthotheca* ($F_{ST} = 0.12$) and *Swietenia macrophylla* ($\Phi_{ST} = 0.28$), meaning that gene flow is restricted across its populations in western Kenya (Alcalá et al. 2015; Bouka et al. 2022). Similar reduction in gene flow due to habitat fragmentation has been reported in plant populations (Cheptou et al. 2017).

The differentiation indices in this study ($\Phi_{ST} \approx 0.35$; $G_{ST} \approx 0.27$) corresponded well with a mean gene flow estimate of $N_m = 2.39$, reflecting moderate connectivity, sufficient to reduce genetic drift but insufficient to homogenize populations completely. This balance demonstrates that the populations of *T. emetica* may be undergoing semi-independent evolutionary processes, with geographic isolation and environmental gradients acting as filters to exchange of genes. Rajarajan et al. (2024) reported comparable trends of genetic differentiation and population structuring that are influenced by environmental heterogeneity in *Azadirachta indica* and *Melia azedarach*, reinforcing the view that ecological variation drives genetic structuring within the Meliaceae. Similar levels of gene flow and spatial structuring have been reported in *Acacia senegal* (Omondi et al. 2023), *Khaya anthotheca* (Bouka et al. 2022), and *Swietenia macrophylla* (Limongi Andrade et al. 2022), where fragmentation and ecological discontinuities have produced moderate differentiation despite ongoing genetic connectivity. Variation in G_{ST} values (0.04–0.46) across ISSR loci further demonstrated unevenness of

genetic differentiation across the genome, differing levels of mutation, selection, or gene exchange among loci. Primers such as UBC-809 and UBC-864, which had intermediate *Gst* values, may have captured genomic regions undergoing partial isolation, while highly differentiated loci like UBC-888 likely reflect regions of genome under selection or low recombination. This trend is in line with findings in *Melia dubia* (Rawat et al. 2018) and *Juniperus excelsa* (Al-Yasi and Al-Qthanin 2024), where ISSR markers effectively revealed localized allelic divergence in response to ecological isolation and restricted pollen flow.

The PCoA and the UPGMA analyses supports these molecular patterns. These analyses showed three different genetic clusters: (i) Siaya–Vihiga–Kisumu, (ii) Bungoma–Kakamega, and (iii) Nandi. The similarity between UPGMA and PCoA underline the strength of these relationships and their ecological basis. The first two principal coordinates accounted for the majority of total variance and delineated populations along ecological gradients from lowland to highland zones, reflecting the regional topography and rainfall patterns of western Kenya.

The *T. emetica* population from Nandi occupied the first coordinate axis and had the highest Nei's genetic distances, ranging from 0.13 to 0.24, and had the least genetic distances of between 0.79 and 0.88 relative to other populations. This variation in classification can be linked to its ecological and geographical isolation in the highlands, where reduced pollen and seed dispersal, coupled with differences in climatic conditions, support genetic divergence. Previous studies showing distinct genetic differentiation connected to topographic and ecological discontinuities has been reported in *Swietenia macrophylla* from fragmented Mexican forests (Alcalá et al. 2015), *Osyris lanceolata* in East Africa (Mugula et al. 2023), and *Parashorea chinensis* across Southeast Asia (Li et al. 2024; Xu et al. 2024).

In contrast, the populations of *T. emetica* from Siaya, Kisumu, and Vihig) displayed very low genetic distances, which ranged from 0.02 to 0.03, and high identity values greater than 0.97, pointing a recent common ancestry or ongoing gene flow preserved through landscape connectivity such as riverine corridors or agricultural mosaics. These findings mirror the weak spatial structuring reported in *Cedrela odorata* across Neotropical lowlands (Finch et al. 2022) and *Sonneratia caseolaris* in coastal Vietnam (Le and Le 2024), where gene flow remains relatively unrestricted in connected habitats. On other hand, Bungoma and Kakamega clustered in a moderately distinct category, likely sustained by partial connectivity through remnant mid-altitude forest corridors. The small Nei's distance of 0.03 observed between these two populations and their close grouping on the dendrogram support the presence of ongoing but reduced gene flow across fragmented forest patches.

Conservation implications of genetic diversity patterns in *T. emetica*

The ISSR genetic patterns in this study have important conservation and sustainable management implications for *T. emetica* populations in western Kenya. The high level of polymorphism (94.65%) and the finding that 65% of the total variation occurs within populations indicate that substantial local genetic diversity still exists. This within-population diversity is vital for maintaining adaptive potential and ensuring long-term evolutionary resilience, particularly under increasing environmental and

anthropogenic pressures. Given projections of shifting climatic zones and increased habitat stress, maintaining such genetic diversity is crucial for forest resilience and adaptive response (Konrad et al. 2025). However, the relatively high population differentiation ($\Phi_{ST} = 0.35$; $G_{ST} = 0.27$) shows that genetic resources are unevenly distributed, highlighting the need for population-specific conservation strategies rather than a uniform management approach. Similar to patterns reported in fragmented alpine and tropical plant systems, where restricted gene flow and local adaptation demand site-specific conservation actions (Cheptou et al. 2017; Soares et al. 2019), these findings emphasize that the management of *T. emetica* should account for regional ecological and genetic distinctiveness.

Populations such as Nandi and Kakamega, which exhibited the highest gene diversity ($H_e = 0.24$ and 0.18 , respectively) and the greatest percentage of polymorphic loci, represent genetic reservoirs essential for the species' survival. These populations should be prioritized as core units for in situ and ex situ conservation, serving as key sources for seed collection and the establishment of genetic resource banks. Similar conservation initiatives integrating molecular diversity and population structure have been conducted for *Vitellaria paradoxa* in Côte d'Ivoire (Attikora et al. 2024), highlighting the importance of conserving genetically diverse populations as reservoirs for breeding and restoration. In contrast, populations from Siaya and Kisumu, which recorded low gene diversity ($H_e = 0.10$ – 0.11) and only 31.25% polymorphic loci, are vulnerable to genetic erosion and stochastic loss. These populations require genetic enrichment through assisted regeneration, enrichment planting, and controlled exchange of germplasm from genetically richer populations to prevent further decline in adaptive capacity (Donkpegan et al. 2020). The moderate fixation index ($\Phi_{ST} = 0.35$) and variable G_{ST} values among ISSR loci (0.04 – 0.46) reflect limited genetic exchange between populations. From a conservation perspective, this underscores the importance of preserving and restoring gene flow corridors between fragmented forest remnants. Conservation planning should prioritize connectivity conservation, linking genetically related populations such as Bungoma-Kakamega and Kisumu -Vihiga -Siaya to sustain pollen and seed dispersal. Establishing community-based forest restoration zones that utilize genetically diverse and locally adapted materials can help stabilize population structures and mitigate ongoing isolation. Furthermore, the clear delineation of populations into three genetic groups: (i) Siaya-Vihiga-Kisumu, (ii) Bungoma-Kakamega, and (iii) Nandi, provides a strong foundation for conservation and seed zoning. Such genetic zoning supports Kenya's forest seed production and certification systems, ensuring that restoration initiatives use materials compatible with the adaptive traits of each ecological region (FAO 2025). Integrating this molecular data into forest restoration policy will enhance seed sourcing, prevent maladaptation, and strengthen reforestation programs aligned with the Bonn Challenge (IUCN 2011), the UN Decade on Ecosystem Restoration (2021–2030), and Sustainable Development Goal 15 (Life on Land) (FAO 2025). Such integration is needed, given evidence that human activities are eroding genetic diversity and threatening species persistence (Soares et al. 2019; Omondi et al., 2023).

Limitations of the study

The present study reports the first molecular data on the genetic diversity and population structure of *T. emetica* in Kenya. The fifteen ISSR markers revealed over 94% level of polymorphism and delineated the

T. emetica populations into distinct clusters that corresponded with ecological stratification. The sample size of 20 individuals per population was adequate to detect genetic diversity but may have been insufficient to fully capture intra and inter population variation. The dominant nature of ISSR markers does not allow distinction between homozygous and heterozygous loci, reducing precise estimation of heterozygosity, inbreeding coefficients, and allelic richness. Since ISSRs target non-coding regions of the DNA, they do not accurately dissect adaptive loci. However, chloroplast genomic studies in Meliaceae have revealed interspecific variation useful for phylogenetic and adaptive inference (Nie et al. 2025), indicating that integrating plastid and nuclear genomic tools could enhance understanding of *T. emetica*'s evolutionary history. In this context, combining chloroplast genomic data with co-dominant nuclear markers like SSRs and SNPs would enable precise estimation of heterozygosity, inbreeding, and adaptive loci (Bouka et al. 2022; Limongi Andrade et al. 2022; Faria et al. 2024), thereby providing a comprehensive view of genetic structure and adaptive potential. Hence, while ISSRs produced valuable baseline molecular data for assessing genetic variation and population differentiation,, integrating plastid and co-dominant markers can help refine the adaptive diversity, evolutionary dynamics, and conservation of *T. emetica*.

Conclusion

The present study dissected the genetic diversity and population structure of *T. emetica*. The molecular analysis uncovered over 94% genome-wide polymorphism, with 65% of total variation occurring within and 35% among populations. The populations of *T. emetica* from Nandi and Kakamega displayed the highest genetic diversity, whereas Siaya and Kisumu had reduced variability. The population differentiation ($\Phi_{ST} = 0.35$) and clustering patterns revealed restricted but ongoing gene flow among populations. These findings provide molecular evidence that *T. emetica* maintains considerable intra-population variation. The enhanced genetic structuring across ecological zones shows the influence of topography, climate, and habitat degradation on gene exchange and adaptive potential. Conservation efforts should prioritize Nandi population as core genetic reservoirs, while enhancing connectivity and regeneration in Siaya and Kisumu populations through assisted natural regeneration and targeted enrichment planting. Despite the contributions made in this study, it used fewer individuals to represent each population and relied on dominant ISSR markers that do not distinguish heterozygotes from homozygotes, reducing the estimation of heterozygosity, inbreeding, and adaptive variation. Future studies should increase the number of individuals sampled per population and incorporate co-dominant or high-resolution genomic tools to capture both neutral and adaptive diversity. Therefore, preserving the genetic diversity of *T. emetica* is critical for sustaining forest ecosystem resilience, supporting local livelihoods, and ensuring adaptive responses under climate change.

Declarations

Compliance with Ethical Standards

This study was exempted from ethical review for human clinical trials or animal experiments, as it involved only collection of plant leaves. The Ethics and Review Committee of the University of Eldoret reviewed and approved the study.

Clinical trial number

not applicable

Consent for publication:

All authors have reviewed and approved the manuscript for submission to the *New Forests*

Competing interests:

The authors declare no competing interests.

Funding

The authors did not receive funding from any organization the submitted work.

Author Contribution

The study was carried out as part of the first author's Master of Science thesis in Plant Genetics at the University of Eldoret. E.K.S: conceptualized the study, designed the methodology, and conducted data collection and analysis, drafted original manuscript. B .O.N and O.G.D: supervised the first author, critically reviewed the manuscript and approved version for submission. K.P.A: Supported the first author in sample collections and reviewed the manuscript.

Acknowledgement

The authors are grateful to Kenya Agricultural and Livestock Research Organization (KARLO) at Njoro for providing laboratory facility to undertake this study. We would like to thank Martin Lagat and Cyrus Kimani from KALRO-Njoro for technical support during the study. We would like to thank Ms. Samantha Cynthia Akinyi for editing the manuscript.

Data Availability

All data supporting the findings of this study are available within the paper and raw data can be obtained upon request from the first author.

References

1. Al-Yasi HM, Al-Qthanin R (2024) Comparing genetic differentiation and variation using ISSR and SCoT among *Juniper* plant markers in Saudi Arabia. *Front Plant Sci* 15:1356917. <https://doi.org/10.3389/fpls.2024.1356917>
2. Aldholmi M, Althomali E, Aljishi F, Ahmad R, Alqathama A, Alaswad D (2024) A comparative study on the antidiabetic activity, cytotoxicity and lipid profile of *Trichilia emetica* oils. *Plants*, 13(16), 2234. <https://doi.org/10.3390/plants13162234>
3. Akweni AL, Zharare GE, Zimudzi C (2021) Predicting the number of fruits and the seed biomass of *Trichilia emetica* (Vahl.) in the eastern coastal region of South Africa. *Trees For. People* 6: 100138. <https://doi.org/10.1016/j.tfp.2021.100138>
4. Alicandri E, Vettraino AM, Agrimi M, Ciaffi M, Kuzminsky E. (2022) Molecular markers dataset to assess the genetic diversity of oriental plane trees from historical sites in Lazio (central Italy). *Data in Brief*, 43, 108100. <https://doi.org/10.1016/j.dib.2022.108100>
5. Alcalá RE, Molina-Freaner F, Piñero D, Oyama K (2015) Genetic diversity and population structure of *Swietenia macrophylla* in fragmented landscapes of Mexico. *For Ecol Manag* 344:1–9. <https://doi.org/10.1016/j.foreco.2015.02.011>
6. Attikora AJP, Yao SDM Dago ND, Silué S, De Clerck C, Kwibuka Y, Diarrassouba N, Alabi T, Achigan-Dako EG, Lassois L (2024) Genetic diversity and population structure of superior shea trees (*Vitellaria paradoxa* subsp. *paradoxa*) using SNP markers for the establishment of a core collection in Côte d'Ivoire. *BMC Plant Biology*, 24, 913. <https://doi.org/10.1186/s12870-024-05055-8>
7. Borah R, Bhattacharjee A, Rao SR, Kumar V, Sharma P, Upadhaya K, Choudhury H (2021) Genetic diversity and population structure assessment using molecular markers and SPAR approach in *Illicium griffithii*, a medicinally important endangered species of Northeast India. *Journal of Genetic Engineering and Biotechnology*, 19, 118. <https://doi.org/10.1186/s43141-021-00211-5>
8. Bouka GUD, Doumenge C, Ekué MRM, Daïnou K, Florence J, Degen B, Loumeto JJ, McKey D, Hardy OJ. (2022) *Khaya* revisited: Genetic markers and morphological analysis reveal six species in the widespread taxon *K. anthotheca*. *Taxon*, 71(3), 637–653. <https://doi.org/10.1002/tax.12720>
9. Chebii WK, Muthee JK, Kiemo JK (2022) Traditional medicine trade and uses in the surveyed medicine markets of Western Kenya. *African Health Sciences*, 22(4), 684–697. <https://doi.org/10.4314/ahs.v22i4.76>
10. Cheptou P-O, Hargreaves AL, Bonte D, Jacquemyn H (2017) Adaptation to fragmentation: Evolutionary dynamics driven by human influences. *Philos. Trans. R. Soc. B Biol. Sci.* 372(1712): 20160037. <https://doi.org/10.1098/rstb.2016.0037>
11. Donkpegan ASL, Piñeiro R, Heuertz M, Duminil J, Daïnou K, Doucet J-L, Hardy OJ (2020) Population genomics of the widespread African savannah trees *Azelia africana* and *Azelia quanzensis* reveals

- no significant past fragmentation of their distribution ranges. *American Journal of Botany*, 107(3), 498–509. <https://doi.org/10.1002/ajb2.1449>
12. Doyle JJ (1991) DNA protocols for plants. In: Hewitt GM, Johnston AWB, Young JPW (eds) *Molecular techniques in taxonomy*. NATO ASI Series, vol 57. Springer, Berlin, Heidelberg, pp 283–293. https://doi.org/10.1007/978-3-642-83962-7_18
 13. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131(2):479–491. <https://doi.org/10.1093/genetics/131.2.479>
 14. FAO (2025) *State of the world's forest genetic resources—2025 update*. Food and Agriculture Organization of the United Nations, Rome.
 15. Faria JCT., Konzen ER, Caldeira MVW, Godinho TO, Maluf LP, Moreira SO, Carvalho CS, Leal BSS, Azevedo CS, Momolli DR, Coelho GTCP, Oliveira CMB, Soares TCB (2024) Genetic resources of African mahogany in Brazil: Genomic diversity and structure of forest plantations. *BMC Plant Biol.* 24: 858. <https://doi.org/10.1186/s12870-024-05565-9>
 16. Finch KN, Jones FA, Cronn RC (2022) Cryptic species diversity in a widespread Neotropical tree genus: The case of *Cedrela odorata*. *American Journal of Botany*, 109(11), 1639–1653. <https://doi.org/10.1002/ajb2.16064>
 17. Forest & Trees, Agroforestry Flagship 1 (2025) *Restoration and biodiversity conservation in forest landscapes*. World Agroforestry (ICRAF), Nairobi.
 18. International Union for Conservation of Nature (IUCN) (2011) *Bonn Challenge: A global effort on restoration*. Gland, Switzerland. <https://www.bonnchallenge.org/>. Accessed 11 Aug 2025
 19. Jaetzold R, Schmidt H, Hornetz B, Shisanya CA (2006) *Farm management handbook of Kenya*, Vol. II. Ministry of Agriculture, Nairobi, Kenya.
 20. Kenya Forestry Research Institute (KEFRI) (2021) *Annual report 2020/2021*. KEFRI Publications, Nairobi.
 21. Kogo BK, Kumar L, Koech R (2019) Forest cover dynamics and underlying driving forces affecting ecosystem services in western Kenya. *Remote Sens Appl Soc Environ* 14:100342. <https://doi.org/10.1016/j.rsase.2019.02.007>
 22. Konrad H, George J-P, Szukala A (2025) The state of forest genetic diversity: Anthropogenic impacts and conservation initiatives. In Lapin K, Oettel J, Braun M Konrad H (Eds.) *Ecological connectivity of forest ecosystems* (pp. 55–90). Springer Nature. <https://doi.org/10.1007/978-3-031-82206-3>
 23. Le S, Le TV (2024) Genetic diversity and population structure of natural provenances of *Sonneratia caseolaris* in Vietnam. *J Genet Eng Biotechnol* 22(1):100356. <https://doi.org/10.1016/j.jgeb.2024.100356>
 24. Li X, Zhang Y, Liu H, Zhang Y, Huang J (2024) Geographical variation and genetic diversity of *Parashorea chinensis* germplasm resources. *Front Plant Sci* 15:1452521. <https://doi.org/10.3389/fpls.2024.1452521>

25. Limongi Andrade R, Pico-Mendoza J, Morillo E, Buitrón J, Meneses S, Navarrete B, Pinoargote M, Carrasco B (2022) Molecular characterization of mahogany tree (*Swietenia macrophylla* King, Meliaceae) in the remnant natural forest of Ecuador. *Neotropical Biodiversity*, 8(1), 222–228. <https://doi.org/10.1080/23766808.2022.2080334>
26. Mabaso M, Buthelezi LG, Zharare G E (2025) Comparative analysis of Mafura butter oils from *Trichilia emetica* and *Trichilia dregeana* extracted by screw-press from seeds collected in KwaZulu-Natal Province of South Africa. *Plants* 14(19): 3071. <https://doi.org/10.3390/plants14193071>
27. Mugula BB, Omondi S, Curto M, Kiboi SK, Kanya JI, Egeru A, Okullo P, Meimberg H (2023) Microsatellites reveal divergence in population genetic diversity and structure of *Osyris lanceolata* in Uganda and Kenya. *BMC Ecol Evol* 23:73. <https://doi.org/10.1186/s12862-023-02182-2>
28. Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89(3):583–590. <https://doi.org/10.1093/genetics/89.3.583>
29. Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* 70(12):3321–3323. <https://doi.org/10.1073/pnas.70.12.3321>
30. Nie Z, Ma J, Wang C, Tang M, Jia T, Liao G, Zhang L (2025) Comparative analysis of chloroplast genomes in Meliaceae species: Insights into evolution and species identification. *Front Plant Sci* 16:1536313. <https://doi.org/10.3389/fpls.2025.1536313>
31. Omondi SF, Githae EW, Khasa DP (2023) Long-distance gene flow in *Acacia senegal*: Hope for disturbed and fragmented populations. *Ecol Evol* 13(9):e10292. <https://doi.org/10.1002/ece3.10292>
32. Osewe EO, Niță MD, Abrudan IV (2022) Assessing the fragmentation, canopy loss and spatial distribution of forest cover in Kakamega National Forest Reserve, Western Kenya. *Forests* 13(12): 2127. <https://doi.org/10.3390/f13122127>
33. Oyedeji-Amusa MO, Sadgrove NJ, Van Wyk B-E (2021) The ethnobotany and chemistry of South African Meliaceae: A review. *Plants* 10(9):1796. <https://doi.org/10.3390/plants10091796>
34. Peakall R, Smouse PE (2012) GenAEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* 28(19):2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
35. PROTA (2025) *Plant resources of tropical Africa (Meliaceae)*. Wageningen University, The Netherlands. <https://prota.prota4u.org/protav8.asp?g=pe&p=Trichilia%20emetica>. Accessed 11 Aug 2025
36. Rajarajan K, Sahu S, Handa AK, Arunachalam A, Priyadarshini E, Bharti A, Yuvaraja A, Muthamilarasn M, Anuragi H (2024) Genetic diversity and population structure of *Azadirachta indica* using simple sequence repeat (SSR) markers for genetic resource conservation and utilization. *Genet. Resour. Crop Evol.* 71(8): 3383-3394. <https://doi.org/10.1007/s10722-023-01841-z>
37. Rawat S, Arunkumar AN, Annapurna D, Karaba NN, Joshi G (2018) Genetic diversity of *Melia dubia* using ISSR markers for natural populations and plantations. *Int. J. Genet* 10(9): 490-494. <https://www.bioinfopublication.org/jouarchive.php?opt=&joid=BPJ0000226>

38. Rotich B, Ojwang D (2021) Trends and drivers of forest cover change in the Cherangany Hills Forest Ecosystem, western Kenya. *Glob Ecol Conserv* 30:e01755. <https://doi.org/10.1016/j.gecco.2021.e01755>
39. Ruiz-González H, Raggio M, Labrín-Sotomayor NY, Ferrer MM, Peña-Ramírez YJ (2023) Genetic variability of *Tabebuia rosea* from plantations and remnant natural populations in the Mayan Forest. *Forests* 14(10):2006. <https://doi.org/10.3390/f14102006>
40. Sheikina OV, Romanov EM (2024) Variability of Scots pine (*Pinus sylvestris* L.) plus trees in the Middle and Upper Volga Region using ISSR markers. *Vavilovskii Zh Genet Selektiv* 28(2):148–154. <https://doi.org/10.18699/vjgb-24-17>
41. Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* 236(4803):787-792. <https://doi.org/10.1126/science.3576198>
42. Soares LASS, Cazetta E, Santos LR, França DS, Gaiotto FA (2019) Anthropogenic disturbances eroding the genetic diversity of a threatened palm tree: A multiscale approach. *Front Genet* 10:1090. <https://doi.org/10.3389/fgene.2019.01090>
43. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729. <https://doi.org/10.1093/molbev/mst197>
44. Tsomele GF, Venter E, Wokadala OC, Jooste E, Dlamini BC, Ngobese NZ, Siwela M (2021) Structural (gross and micro), physical and nutritional properties of *Trichilia emetica* and *Trichilia dregeana* seeds. *CyTA J. Food* 19(1): 483–492. <https://doi.org/10.1080/19476337.2021.1915877>
45. Uras ME, Filiz E, Sen U, Ozyigit II (2024) Genetic diversity and phylogenetic analysis of *Robinia pseudoacacia* L. populations using ISSR markers, ITS1 and *trnL-F* intergenic spacer sequences. *J For Sci* 70(1):1–13. <https://doi.org/10.17221/95/2023-JFS>
46. Viswanathan MBG, Rajasekar C, Sathish Kumar P (2018) ISSR and ITS analyses to assess genetic diversity and phylogeny to conserve an endemic and critically endangered tree, *Memecylon subcordatum*, in India. *Ecol. Genet. Genom.* 7–8:6–12. <https://doi.org/10.1016/j.egg.2018.03.004>
47. Xu Y, Liu S, Finnegan PM, Liu F, Ali I, Zhang H, Yang M (2024) Geographical variation and genetic diversity of *Parashorea chinensis* germplasm resources. *Front Plant Sci* 15:1452521. <https://doi.org/10.3389/fpls.2024.1452521>
48. Yeh FC, Yang RC, Boyle TJB, Ye Z, Mao JX (2000) *POPGENE version 1.32: Microsoft Windows-based freeware for population genetic analysis*. University of Alberta and Centre for International Forestry Research, Edmonton. <https://www.scienceopen.com/book?vid=2d45ad78-b140-4b66-b80f-2c9f513ec997>. Accessed 11 Jun 2025

Figures

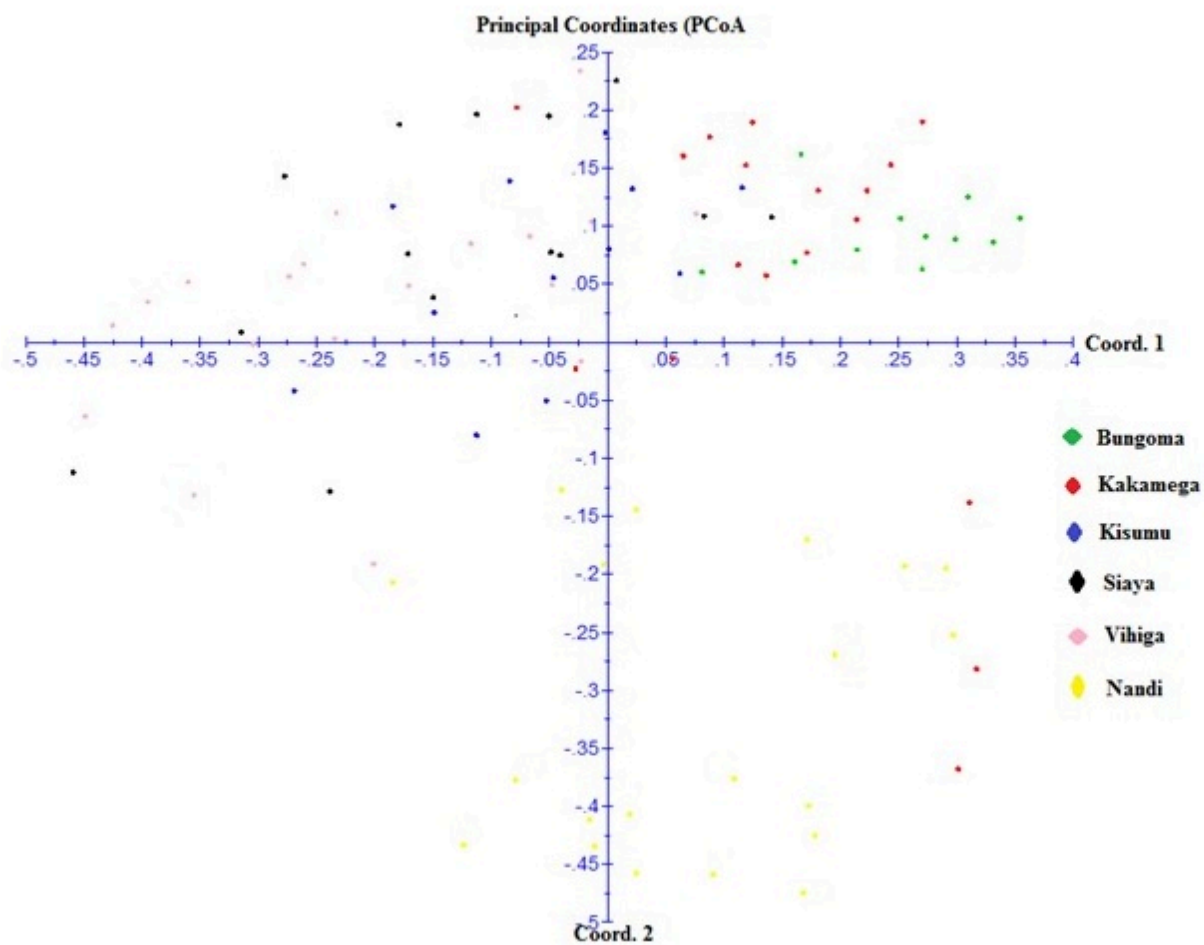


Figure 1

Principal coordinates analysis showing genetic relationships among 120 individuals representing six *T. emetica* populations based on ISSR data



Between	And	Length
Vihiga	Siaya	1.95109
Siaya	Kakamega	4.80742
Kakamega	Bungoma	1.30751
Siaya	Kisumu	4.74127
Kisumu	Bungoma	0.19252
Bungoma	Kisumu	1.18115
Bungoma	Vihiga	1.18115
3Kisumu	Siaya	1.37367
5Vihiga	Nandi	8.06603

Figure 2

UPGMA dendrogram constructed from Nei's unbiased genetic distances illustrating genetic relationships among six *T. emetica* populations in western Kenya based on ISSR marker data