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# Phenotypic Variation of Four Populations of *Osyris lanceolata* Hochst. & Steud. (African Sandalwood) in Kenya

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**Abstract**

*African sandalwood (Osyris lanceolata) is a dioecious and semi-parasitic tree at risk of extinction due to overexploitation for its essential oil, a product used in the perfumery and pharmaceutical industries. This study therefore determined the level of morphological variation in four kenyan populations of O. lanceolata, namely Gwasii, Kabarnet, Marigat, and Kitui, with the objective of recommending conservation strategies for the species. morphological traits were analysed using Anova and coefficient of variation. genetic similarity was analysed using a multivariate analysis using euclidean distance and a dendrogram constructed based on unweighted pair group method with arithmetic mean (UPGMA). Results showed that, Gwasii trees had significant higher diameter at breast height (dbh), crown diameter (crd), and flower diameter (fld) than the rest. female trees had greater growth and floral mean values than male trees as observed in tree height (het), dbh, crd, flower length (fll) and fld. Analysis based on euclidean distance clearly distinguished Gwasii population from the Kitui, Kabarnet, and Marigat populations, which individuals revealed genetic interrelationship. in conclusion, morphological traits revealed great individual interspersion within and among populations revealing population genetic interrelationships. Study recommended that Gwasii population should be prioritized for ex-situ conservation while in-situ conservation should target plus trees in entire populations.*

**Keywords**: Conservation, Morphological Variation, *Osyris lanceolata*

## INTRODUCTION

*Osyris lanceolata* Hochst. & Steud. also commonly known as the East African Sandalwood belongs to the family Santalaceae. It is an evergreen shrub, which can reach a height of 10 m at maturity in favorable forest conditions. The diameter at breast height (DBH) of mature trees ranges from 3.5 to 6 cm, although some trees may reach a diameter of 11.5 cm in very fertile soils (Teklehaimanot et al., 2003; Mwang'ingo et al., 2007). The species is a multi-stemmed, dioecious shrub that can produce up to seven leader stems per tree, mostly ranging from two to four stems in both male and female trees (Teklehaimanot et al., 2003).

It has been documented to grow in Nepal, Algeria, Eritrea, South Sudan, Rwanda, Burundi, Somalia, Zambia, Malawi, Mozambique, Zimbabwe, and South Africa (Mwang'ingo et al., 2007; CITES, 2016; da Silva et al., 2016) and also occurs in the three East African countries of Kenya, Uganda, and Tanzania (Teklehaimanot et al., 2003; Mwang'ingo et al., 2007; Machua et al., 2009). Literature reveals that several genera of the Santalaceae family grow naturally in

Ethiopia, Australia, Asia (India and China), Pacific Islands, Europe (the Iberian Peninsula and Balearic Island), Port Vila (Vanuatu), and Socotra (Butaud et al., 2005; Hudson, 2008; Page et al., 2020; Bush et al., 2020; Khan et al., 2021; Erbo & Awas, 2021).

*Osyris lanceolata* is widely distributed in Kenya, from the hilly areas of Amboseli, Loitoktok, Kibwezi (Chyullu Hills), Taita Hills, Ngong Hills to the drylands of Narok, Mbeere, Kitui, Mau, Kajiado, West Pokot, Makueni, Gwasii Hills, Turkana, Laikipia, Marsabit, Koibatek and Meru North Districts (Mathenge et al., 2005; Kamondo et al., 2014; CITES, 2016). It is a hemiparasitic tree, which survives on roots of host trees such as *Searsia natalensis* (Bernh. ex C. Krauss) F. A. Barkley, *Dodonaea viscos*a (L.) Jacq., *Tecomaria capensis* (Thunb.) Spach, *Catha edulis* (Vahl) Endl. *Apodytes dimidiata* E.Mey. ex Arn., *Brachystegia spiciformis* Benth., *Pongamia pinnata* (L.) Pierre, *Casuarina equisetifolia* L., and *Aphloia theiformis* (Vahl) Benn. (Teklehaimanot et al., 2003; Mwang'ingo et al., 2007). Other hosts observed in the Kenyan populations include *Cupressus lusitanica* Mill., *Dodonaea viscosa* subsp. *angustifolia* (L.f.) J. G. West, *Acacia gerrardii* Benth., *Combretum molle* R.Br. ex G. Don, *Ziziphus mucronata* Willd. and *Lantana camara* L. (Ma et al., 2005; Kamondo et al., 2014).

*Osyris lanceolata* is highly exploited for its valuable and diverse products that include essential oils for making perfumes and expensive cosmetics, herbal medicines from back and roots, and carvings just like other sandalwood trees in the family (Beentje, 1994; Mwang'ingo et al., 2007; Tshisikhawe, 2012; Subasinghe, 2013). Overexploitation is exacerbated by the high demand for its products in the world market (Kamondo et al., 2014). The declining sandalwood populations in other countries has led to rising cases of illegal harvesting by smugglers from the natural forests in Kenya, Tanzania, Uganda, and other Eastern Africa countries despite restrictions by the various governments (Brand, 1999; Teklehaimanot et al., 2003; Mwang'ingo et al., 2007; Kamondo et al., 2014).

Although protected by Legal Notice No. 3176 of 2007 under the Forests Act, 2005 (Kenya Law, 2007), *O. lanceolata* populations in Kenya have been decreasing since 2002 because of a sharp rise in the illegal extraction of the species for international trade (Mwang'ingo et al., 2003; Mwang'ingo et al., 2007; Kamondo et al., 2014). Kenya has made several applications for the species to be included in the IUCN Red list and it is being debated for inclusion in the "threatened species" category of the IUCN Red List to foster its conservation. The Australian, Asian (India and China), and Pacific Islands species *Santalum insulare* (Bertero ex A. DC.) are listed as a 'vulnerable species' by the IUCN because of overexploitation and low populations in the wild (Bunei, 2017). The Australian *Santalum spicatum* (R.Br.) A.DC is currently protected by the governments and is extensively planted in farmlands and protected areas to counter impact of overexploitation (Moniodis et al., 2018).

The phenotypic variations and phylogenetic structure of *O. lanceolata* populations in Kenya have not been studied (Kamondo et al., 2014). While it is desirable to employ both morphological and molecular markers to estimate genetic diversity, morphological characterization is preferable as the first step because it is inexpensive and can be easily implemented (Peng et al., 2014; Zhang et al., 2015). In addition, morphological traits of trees are also used to describe their life cycles, geographical and ecological distributions, conservation status, delimit species as well as help to infer environmental effects on the species (Doležal et al., 2004). This paper reports the genetic variation of *O. lanceolata* based on a set of growth, floral, and fruit traits to provide information that can be used in the development of appropriate conservation strategies.

## MATERIALS AND METHODS
### Description of the Study Area
The assessment of morphological traits was done separately on 24 individual trees per population in four distinct populations occurring in Gwasii (00° 30' S, 34° 10' E), Marigat (00° 50' N, 35° 58' E), Kabarnet (00° 10 'N, 34° 43' E) and Kitui (01° 21' S, 38° 00' E), which represent the natural distribution areas of the species in Kenya (figure 1). The eco-climatic site data of the areas where *O. lanceolata* populations were sampled are given in table 1.

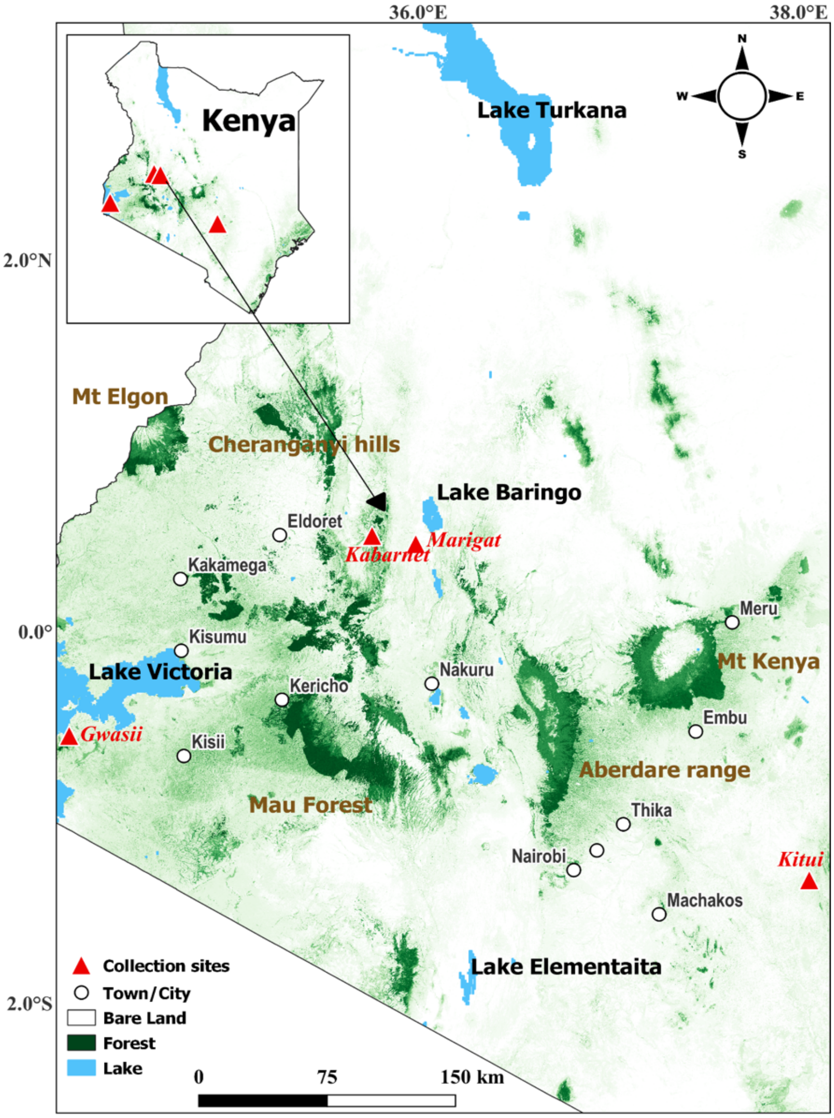


**Figure 11: Location of populations of *O. lanceolata* sampled.**

**Table 1: Eco-climatic site data of the areas where *O. lanceolata* populations were sampled**

| Population | Eco-zones[1] | Longitude | Latitude | Altitude (m) | M. A. Rainfall (mm) | M. A. Temperatures (˚C) |
|---|---|---|---|---|---|---|
| Gwasii | IV | 34˚ 10'E | 00˚ 30' S | 1600.7 | 1100 | 26.0 |
| Kabarnet | V | 34˚ 43'E | 00˚ 10' N | 2007.4 | 813 | 24.5 |
| Marigat | V | 35˚ 58'E | 00˚ 50' N | 2040.0 | 635 | 25.0 |
| Kitui | V | 38˚ 00'E | 01˚ 21' S | 1185.4 | 775 | 25.0 |

[1] : Eco-zone V (semi-arid) - Mean annual rainfall (MAR) range-450-900, mean annual temperature (MAT) -18-22: [2] : Eco-zone IV (semi humid) - MAR range- 600-1000, MAT range- 16-18

**Assessment of Morphological Traits**

Twenty-four mature trees growing at least 50 m apart were selected and marked within each of the four populations. Nine (9) morphological characteristics were assessed. The following four growth parameters were measured: tree height (HET) in meters (m) using a tree height bole; tree diameter at breast height (DBH) in centimeters (cm) using a tree diameter tape, tree crown diameter (CRD) measured in meters (m) using a linear tape for two crosswise diameters and averaged, and the number of leader stems (STC) directly counted. Ten flowers from every marked tree in each population were collected and stored in labelled glass bottles containing Formalin Acetylic Alcohol (FAA) preservative and taken to the laboratory for assessment. Two quantitative parameters from the flowers, the length (FLL) and diameter (FLD) of male and female flowers were measured using a millimeter ruler. Fifty mature fruits were randomly selected from the 24 previously marked trees in every population. The fruits were stored in labelled universal bottles containing Formalin Acetylic Alcohol (FAA) preservative and taken to the laboratory for measurement of the fruit length (FRL) and fruit diameter (FRD). Four replicates of the 1000 fully formed fruits samples per population were extracted and oven-dried at 36° C to constant weight. Seed weight was expressed in grams as 1000-seed dry weight (DSW).

**Data Analysis**

The differences in the quantitative traits among the populations were tested for normal distribution and then analyzed using one-way ANOVA in IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA). Duncan's Multiple Range Tests (DMRT) were performed on DBH, CRD, FLL and FLD morphological parameters only because they proved significant at $p<0.05$, to compare treatment mean differences at $p<0.05$. The within and between population variations were analyzed based on the percentage Coefficient of Variations (CV %). Morphological similarity was based on Euclidean distance in a multivariate analysis were used to construct a dendrogram using unweighted pair group method with arithmetic mean (UPGMA).

## RESULTS

**Morphological Variation**

The results of the analysis of nine morphological parameters measured on trees in the four *O. lanceolata* populations are presented in table 2. The CV was highest in CRD (51.40%) and lowest in DSW (2.21%). Female trees had greater growth (HET, DBH, and CRD) and floral (FLL and FLD) mean values than male trees (table 3). The percentage coefficient of variation revealed that the Gwasii population had the highest population variation (CV=39.76%) followed by Kabarnet (CV=35.80%) with Kitui having the least (CV = 19.64%). The mean percentage coefficient of variation for combined parameters among the populations was 28.79%.

**Table 2: Ranges, Means, Standard deviation (SD) and Coefficient of Variation (CV %) of quantitative traits of *O. lanceolata* assessed in four populations**

| Parameters | Range | Mean | SD | CV % |
|---|---|---|---|---|
| HET (m) | 2.00-6.30 | 3.81 | 1.21 | 31.75 |
| DBH (cm) | 2.10-9.34 | 4.52 | 2.30 | 50.88 |
| CRD (m) | 1.00-4.90 | 2.51 | 1.29 | 51.40 |
| STC (n) | 1.00-5.00 | 2.44 | 0.78 | 32.00 |
| FLL (mm) | 4.00-5.20 | 4.76 | 0.17 | 3.57 |
| FLD (mm) | 2.90-3.90 | 3.63 | 0.34 | 9.37 |
| FRL (mm) | 10.10-10.90 | 10.4 | 0.41 | 3.94 |
| FRD (mm) | 9.20-10.80 | 9.9 | 0.36 | 3.64 |
| DSW (g) | 85.7-93.82 | 90.64 | 2.00 | 2.21 |

**KEY:** HET = height; DBH = diameter at breast height; CRD = crown diameter; STC= stem counts; FLL= flower length; FLD = flower diameter; FRL= fruit length; FRD = fruit diameter; DSW= 1000 seed dry weight).

**Table 3: Comparison of quantitative variables between female (F) and male (M) *O. lanceolata***

| Parameter | Sex | Mean | SD | SE |
|---|---|---|---|---|
| **HET** | F | 3.849 | 1.098 | 0.158 |
| | M | 3.774 | 1.063 | 0.153 |
| **DBH** | F | 4.707 | 2.289 | 0.330 |
| | M | 4.334 | 1.828 | 0.264 |
| **CRD** | F | 2.677 | 1.028 | 0.148 |
| | M | 2.343 | 1.030 | 0.149 |
| **STC** | F | 2.630 | 0.866 | 0.125 |
| | M | 2.250 | 0.700 | 0.101 |
| **FLL** | F | 4.985 | 0.295 | 0.042 |
| | M | 4.527 | 0.672 | 0.096 |
| **FLD** | F | 3.226 | 0.400 | 0.058 |
| | M | 3.147 | 0.238 | 0.034 |

KEY: HET= height; DBH= diameter at breast height; CRD= crown diameter; STC= stem counts; FLL= flower length; FLD = flower diameter; SD = standard deviation; SE= standard error

The percentage coefficients of variation of combined parameters per population were as follows: Gwasii, 39.76%; Kabarnet, 35.80%; Kitui, 19.96%, and Marigat, 19.64%. The ANOVA results showed significant variation at $p < 0.05$ for DBH, CRD, FLL, and FLD (table 5). According to DMRT (table 6), the Gwasii population had significantly higher means ($p<0.05$) for DBH, CRD, FLL, and FLD than the other populations.

**Table 4: ANOVA for nine morphological parameters of *O. lanceolata* in the four populations studied**

| Parameter | Sum of squares | Df | F | *P* |
|---|---|---|---|---|
| **HET** | 5.860 | 3 | 1.728 $^{ns}$ | 0.167 |
| **DBH** | 196.043 | 3 | 28.556* | 0.000 |
| **CRD** | 39.877 | 3 | 19.622* | 0.000 |
| **STC** | 3.375 | 3 | 1.777 $^{ns}$ | 0.157 |
| **FLL** | 15.059 | 3 | 30.239* | 0.000 |
| **FLD** | 6.179 | 3 | 46.529* | 0.000 |
| **FRL** | 0.954 | 3 | 0.110 $^{ns}$ | 0.998 |
| **FRD** | 0.352 | 3 | 0.005 $^{ns}$ | 0.990 |
| **DSW** | 228.061 | 3 | 0.420 $^{ns}$ | 0.989 |

KEY: HET= height; DBH= diameter at breast height; CRD= crown diameter; STC= stem counts; FLL= flower length; FLD = flower diameter; FRL= fruit length; FRD= fruit diameter; DSW = 1000 seed dry weight. * - significant parameters. $^{ns}$= parameters not significant.

**Table 5: DMRT for the four significant morphological parameter at (P<0.05) within the four populations studied**

| | Population | | | | |
|---|---|---|---|---|---|
| **Parameter** | **Gwasii** | **Kitui** | **Kabarnet** | **Marigat** | **SE** |
| **DBH** | $6.993^{b}$ | $3.597^{a}$ | $3.748^{a}$ | $3.743^{a}$ | 0.211 |
| **CRD** | $3.611^{b}$ | $2.052^{a}$ | $2.063^{a}$ | $2.313^{a}$ | 0.106 |
| **FLL** | $5.114^{c}$ | $4.217^{a}$ | $5.157^{c}$ | $4.538^{b}$ | 0.058 |
| **FLD** | $3.627^{b}$ | $2.971^{a}$ | $3.096^{a}$ | $3.092^{a}$ | 0.034 |

*Mean values in a row followed with the same letter (superscript) are not significantly different according to DMRT (P<0.05). DBH, diameter at breast height; CRD, crown diameter; STC, stem counts; FLL, flower length; FLD, flower diameter; SE, standard error.

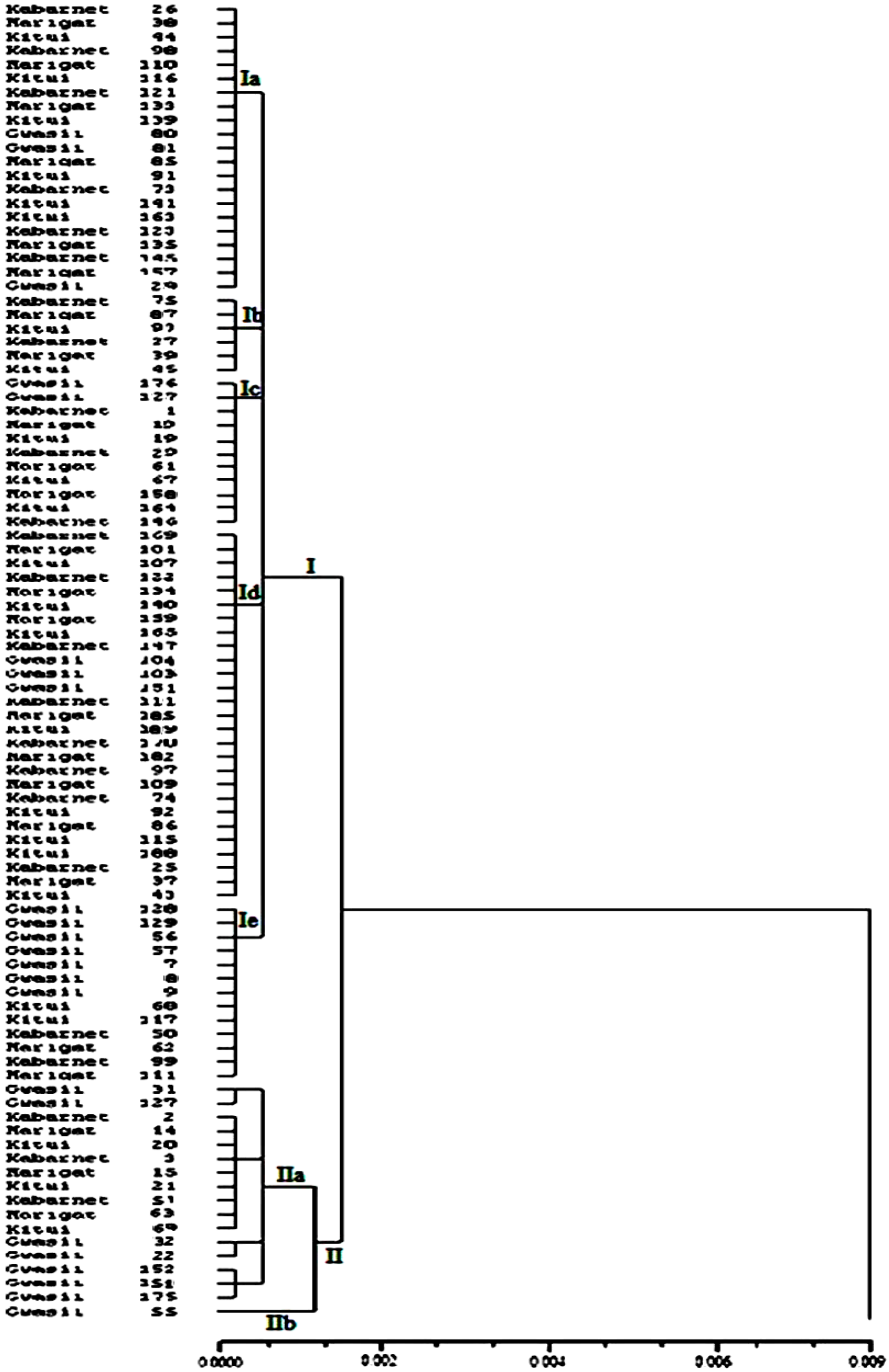


**Figure 2: A UPGMA dendrogram based on Euclidean distances of nine morphological parameters of O. *lanceolata.***

The morphological cluster analysis of tree height, tree diameter at breast height, tree crown diameter, and number of leader stems, flower length, flower diameter, fruit length, fruit diameter, and seed weight showed two distinct clusters among all studied populations (figure 2). The individuals in the four populations clustered in two main groups i.e. I and II. The subdivisions in group I include Ia, Ib, Ic, Id and Ie, and in group II were IIa and IIb. Most of Gwassi individuals like 104, 105, and 151 are sandwiched between Kabarnet population individuals 147 and 171, Gwasii 7, 8, 9, 56, 57, 128, and 129 are sandwiched between Kitui 43, and Kitui 68 and 117 individuals, while Gwasii 32, 33, 55, 152, 153, and 175 occur after a Kitui 69. This implies that many Gwasii individuals are clustered together compared to individuals in Kabarnet and Marigat populations. Only 141 and 163, and 68 and 117 of Kitui individuals and 146 and 169 of individuals in Kabarnet clustered together. Many Kabarnet, Marigat and Kitui individuals intermingle with one another following that order. For instance, Kabarnet 26 is followed with Marigat 38, and then Kitui 44. The sequence repeats with individuals Kabarnet 98, Marigat 110, Kitui 116, and Kabarnet 121, Marigat 133, Kitui 139. This implies that Kabarnet, Marigat and Kitui populations are closely associated genetically.

## DISCUSSION

### Morphological Variation

Cluster analysis showed that the individuals interspersed with one another within and among populations showing inter- and intra-population genetic relationship. Kabarnet, Marigat and Kitui populations of *O. lanceolata* were morphologically closer to one another compared to the Gwasii populations. The close ecological similarity between Kabarnet, Marigat and Kitui may have had a bearing on the overall morphological similarity of the trees in the three populations. The Kabarnet, Marigat and Kitui populations are in eco-zone V (semi-arid) and occur between 1100 and 2050 m a.s.l. The areas where they occur experience rainfall of between 600 mm and 850 mm annually and an annual mean temperature of about 25˚C). It is likely that climatic factors are the main drivers of the observed morphological similarities among the three populations.

The natural distribution of *O. lanceolata* in Kenya shows that it occurs in a wide range of temperature and rainfall regimes (Maundu & Tengnas, 2005). It is therefore expected that the distribution of genetic variation in *O. lanceolata* would follow geographic and climatic factors like temperature, precipitation, growing season length, photoperiod, and biotic agents as observed in other species (Lasky et al., 2013; McKown et al., 2014). These may be important factors for the Gwasii population, which had big trees as depicted by the mean DBH and CRD values, although it was not centre of research in this study. The windward side of Gwasii hills (slope facing Lake Victoria) receives a mean annual rainfall of about 1400 mm that is well distributed all year round (Bradley et al., 2015). Large population of *O. lanceolata* trees grow on the windward side of Gwasii hills and therefore experience conditions, which are conducive for tree growth. Gwasii is generally wetter than Kabarnet, Marigat and Kitui. Therefore, availability of adequate moisture and the fact that *O. lanceolata* trees growing in Gwasii hills are largely inaccessible, could be the reason for their robust growth. The inaccessibility of Gwasii hills has mitigated anthropogenic disturbances and exploitation of *O. lanceolata* trees. The trees therefore have sufficient time and favourable ecological requirements for massive growth. In contrast, *O. lanceolata* trees growing in Kitui were generally small as expected for trees growing in harsh environmental conditions (Athena et al., 2014). The region experiences low annual rainfall (<500 mm) and high annual temperatures (25° C), which probably limits tree growth rates and cause the trees to develop mechanisms for survival under such harsh environmental conditions. In Pyuthan District in Nepal, Poudel *et al*., (2021) studied growth performance and income potential of *Santalum album* L., and noted that site quality and age of a tree were significant in the incremental growth rates of height, diameter, and volume. In a study to investigate growth quality of *S. album* in forests and plantations in Timor Island, Seran et al. (2018) noted that growth performance of trees differed spatially in the studied sites. This implies that some sites favor the rapid growth of the trees than others.

The populations showed higher levels of within-population than between population morphological variations concurring with observations made by Andiego et al. (2019) in the same species using ISSR molecular markers. Mating system of a species is important in determining the genetic diversity of the species (Last et al., 2014; Teixeira et al., 2014). High within population variability implies that the species is out-crossed. This is favoured by the deciduous nature of the species and varied flowering time (Mwang'ingo et al., 2007). High diversity implies a high incidence of out-crossing within the populations, which is likely promoted by the availability of agents of pollination and a healthy sex ratio (Mwang'ingo et al., 2007). Gwasii population was the most morphologically diverse population followed by Kabarnet and the least variable being Kitui. High variation was observed in the trees' crown diameter while dry seed weight showed the least variability. This might have been influenced by age differences, ecological factors, and/or genetic makeup. Genetic makeup, geographical distribution range, and influence from edaphic factors are known to cause morphological variation in tree species (Jones et al., 2009; McKown et al., 2014). In *S. album*, Krishnakumar et al. (2017) noted that height was the growth parameter with the highest heritability. They concluded that high heritability signified high variability which showed influence of environmental factors on complex quantitative tree traits (Krishnakumar et al., 2017). However, in this study high variability was observed in growth traits. This agrees with a previous study by Andiego et al. (2019) which observed high genetic variability in populations of *O. lanceolata*. The selection of superior trees for use in tree conservation and improvement programs often depends on the superiority of phenotypic variability observed in the candidate trees (Jones et al., 2009). In this case, the Gwasii population can be singled out from the other populations. However, other populations might also have important traits that may need consideration in any *O. lanceolata* conservation program. In a study to investigate the association between genetic and environmental factors with essential oil composition in *S spicatum,* Moniodis et al. (2018) observed that geographic distance influenced both morphological and genetic parameters. In *S. album*, Fatima et al. (2019) observed that wide geographic variations influenced morphological variation. The presence of varying levels of host species and haustorial interaction is also known to influence tree accessibility to water and nutrients (Meng et al., 2021). This is a factor that may also be influencing morphological variation among and within O. *lanceolata* populations as observed in *Rhinanthus angustifolius* (Jonstrup et al., 2016). This agrees with the observation that a wide geographical range causes plants to develop adaptive morphological modifications to fit in their habitat, which plays a key role in ecotype development (Dangasuk & Panetsos, 2004; Chiveu et al., 2009; Jonstrup et al., 2016).

Kenyan *O. lanceolata* is multi-stemmed but in this study, the number of stems produced per tree did not vary significantly between populations. The number of stems per tree in each population ranged from one to five with an average of two stems per tree. Trees in Gwasii had the least number of stems while the Marigat and Kabarnet populations had stem counts higher than trees in the Kitui population. A similar study carried out in Tanzania revealed that the number of leader stems produced per tree in *O. lanceolata* varied significantly between populations, ranging from two to six with a mean number of three per tree (Mwang'ingo et al., 2003).

In Gwasii, trees were large with fewer stem count. This agrees with the observation that trees tend to concentrate on secondary growth rather than the production of many leader stems and hence attain a larger DBH (Wallace & Rundel, 1979; Popp & Rinartz, 1988; Riba-Hernández et al., 2016).

**Variation of Morphological Traits among Male and Female Trees**

The comparative analysis of morphological traits revealed that female trees were dimensionally larger than the male trees among the Kenyan *O. lanceolata* populations. This observation shows that perhaps female trees developed mechanisms to compensate for the extra energy required for reproduction and physiological activities (Wallace & Rundel, 1979; Popp & Rinartz, 1988; Riba-Hernández et al., 2016). They develop a large leaf area that assists in photosynthesis and the accumulation of extra energy for their reproductive processes. Mwang'ingo et al. (2010) observed the same in Tanzanian *O. lanceolata* populations. Large morphological dimensions enabled female trees to accommodate an extra load of flowers and fruits (Wallace & Rundel, 1979; Riba-Hernández et al., 2016). This implies that *O. lanceolata* trees in the Gwasii population were better adapted for reproductive functions because of their large sizes compared to trees in other populations. Reproductive success of the populations should therefore be compared to help reinforce this observation.

## CONCLUSION AND RECOMMEDATION

The morphological data provided here shows that the Kitui, Kabarnet and Marigat populations are genetically closer to each other than they are to the Gwasii population. The Gwasii population has trees that are bigger and taller, with bigger flowers and fruits but fewer leader stems compared to the other three populations, making it quite distinct from the others. In all populations, female trees were comparatively larger than male trees. Environmental factors like rainfall, altitude and annual temperature appear to influence tree growth parameters. This study therefore recommend that *in-situ* conservation is required for all the four populations studied due to their varied uniqueness while *ex-situ* conservation should be adopted to conserve any rare phenotypic expressions observed in the populations in general and Gwasii in particular, especially tree height, large diameter at breast height, and fewer but large leaders stems.

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