The influence of seed transfer distance on the growth of *Calotropis procera* (ait) provenances in dry lands of South Eastern Kenya

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Abstract

In a study to domesticate the species *Calotropis procera*, for wool production in drylands, investigations were done to determine the effects of seed transfer distance on the growth of the species in a typical farm setting. The objectives of the research were to determine the growth of three provenances of *C. procera* and to determine the influence of seed transfer distance on the growth parameters. Diameter and height data were used to generate horizontal and vertical growth curves respectively and further subjected to Duncan Multiple Range Test (DMRT) to isolate existence of significant differences across the three provenances. Pearson correlation analysis was used to establish existence of relationships across the growth parameters. Results showed that *C. procera* is a multi-stemmed plant. Duncan Multiple Range Test (DMRT) showed no significant statistical differences (p<0.5) in diameter growth though Pearson correlation analysis, showed strong positive correlations (Pearson, p<0.01) between branching and DBH Test of homogeneity of variances showed significant statistical differences (p<0.01) in vertical growth. The study concluded that *C. procera* is a multi-stemmed plant that can reach a height of 4.48m and a diameter of 7.4cm in two years. It is recommended that *C. procera* seeds for raising nursery stock should be sourced from the nearest source possible. This will reduce the environmental and climatic effects associated with long distance seed transfers and ensure the species benefits from home-site advantages.

Keywords: *Calotropis procera*; Seed transfer distance; Provenance; Vertical; Horizontal growth

1. Introduction

The seed transfer distance is mainly employed in development of seed transfer guidelines in many countries especially for industrial plantations. According to Wani M et.al, [1] seed germination parameters are an important criterion in selection of geographic seed sources for raising stock for bulk commercial forest tree plantations. The seed origin has also been known to determine field growth performance of a provenance [2]. Seed transfer guidelines give local provenances priority and ensure that seeds from a given provenance are not moved over long distances that can lead to pronounced changes in elevation and latitude. The elevation and latitude are key determinants of climate of a given locality through their influence on temperature. According to Matias L et.al. [3], changes in climatic conditions alter growth, survival and physiological performance of seedlings. The influence of latitude on provenance performance has been documented by [4, 5, 6]. Further, [7], in a study on seedlings growth of two provenances of *Nothofagus glaucum*
(Lophozonia glauca), attributed the observed differences to geographical variables, of which latitude was the most important.

To reduce the influence of seed transfer distance, local provenances are given the first priority in afforestation and restoration programmes. [8, 9] stresses on the importance of observing provenance seed transfer distance during restoration activities. [10] investigated how far a plant material should be moved from home sites and remain ecologically appropriate in a restoration setting. Local provenances are known to enjoy home-site advantages compared to foreign provenances. The influence of home-site advantages on provenance performance primarily due to local climatic and environmental conditions have been documented by [11, 12, 13, 14, 15, 10, 8]. Further, [16, 6] documented provenance performance based on environmental conditions associated with seed transfer distances. According to [17], microsite homogeneity is important in seed transfer. [9] reported local provenance as the threshold at which geographic distance corresponds to statistically significant genetic distance. Longer seed transfer distance affects plant productivity and may increase incidences of defects. For instance, a study by Viherä-Aarnio A [5] found out that longer transfer from the south, and transfer from the north, decreased the yield and the proportion of trees with a stem defect increased linearly in relation to the latitudinal seed transfer distance from the south.

In a broad study to domesticate Calotropis procera (Aiton) W.T (Asclepiadaceae) in drylands of South Eastern Kenya for production of wool, investigations were done to determine the influence of seed transfer distance on the growth of three provenances of C. procera. Broadly, C. procera is a xerophytic perennial shrub or small tree that grows in many arid and semi-arid countries [18]. According to Farahat E et.al. [19], the species is a perennial xerophytic medicinal shrub or small tree that grows as a wasteland plant and reproduces by seeds. [20] describes the species as a perennial Asian shrub that primarily reproduces via seeds. According to Galal T et.al. [21], the species is a hardy xerophytic plant which is distributed globally in many countries and has important economic and ecological functions. According to [22, 21], it has a wide range of uses such as medicinal, bark and latex are used in brewing and to curdle milk. Young pods used for fodder, stems produce good charcoal, fibre from stem and white silky tufts, latex or rubber for tannin or dyestuff, poison for arrows and spears, soil fertility, pollution control by monitoring sulphur dioxide emissions in the air, suitable indicator of exhausted soil and plays a critical role in removal of heavy metals from the soil.

2. Material and methods

2.1. Study Site

2.1.1. Geography

![Figure 1 Location of research site in SEKU, Kitui County, Kenya](image-url)
The study was carried out at South Eastern Kenya University (SEKU) situated in Kitui County. The research site is located 15 Kilometers off Kwa Vonza Market, along the Kitui-Machakos main road, Kwa Vonza/Yatta ward, Lower Yatta, Kitui County. Geographically, the research plot lies at 01.31358°S, 037.75546°E and 01.31422°S, 037.75576°E at a general elevation of 1173m a.s.l (Figure 1).

2.1.2. Climate of the study site

The climate of the study areas is semi-arid (Agroecological zone IV) with very erratic and unreliable rainfall. The rainfall pattern is bimodal with the short rainy season occurring between November and December and the long one between April and May. The short rains are more reliable than the long rains [23]. The mean annual rainfall ranges between 500-1050 mm with 40 per cent reliability. The site experiences high average temperatures throughout the year, which range from 16 °C to 34 °C (Pauw et al., 2008). The hot months are between January and February and June and September characterized by mean minimum and maximum temperatures of 28 °C and 32 °C, respectively.

2.1.3. Soils and Geology

Soils are predominantly sandy to loamy sand texture, hence they are susceptible to erosion and are limited in their capacity to retain water and nutrients. The major soil type of the area is lixisols (red soils). Alluvial deposits (fluvisols) occur in isolated patches along rivers and on hill slopes. The soils are generally poorly drained and easily eroded by runoff [24]. Some patches of the research area are overlain by red well drained sandy loam soils which have quartz and feldspar grains and felsic gravel rock fragments. Soil depths (thickness) vary from between 1.2m (upslope) to nearly 2.0m at the downslope side of Mwitasyano stream. The soils reduce in thickness upslope where rock outcrops are found jutting above the surface of the soils. The study site has a similar geology composed of high grade regional metamorphic granitoid granulites which are composed of quartz and feldspars (over 90%) and mafic hornblende and pyroxenes (about 10% or less).

2.2. Selection of the Study Site

The South Eastern Kenya University (SEKU) study site was selected based on the following criteria. Firstly, the site represented typical semi-arid conditions that characterize the larger Kitui County and other dry lands in the country. Secondly, the study required that nursery and field experiments be undertaken. The SEKU study site has existing tree nursery with enough space and other requirements for setting up nursery and field experiments.

2.3. Selection of C. procera Provenances

To capture the dry land conditions in the country, seeds were collected from three areas in Kenya: Baringo, Kibwezi and Tharaka Nithi. Though the three areas are all drylands, they represent different geographical zones within the Country.

2.4. Field Experimental Design

Monitoring of growth of C. procera was carried out in a typical farm setting. A 60m by 80m plot was cleared and levelled. Within the main plot, 27 subplots were demarcated. The subplots were laid out in a randomized complete block design within the main plot. In each of the subplots, 12 planting pits (1ft by 1ft) were dug but at different spacing. The seedlings were transplanted and watering was done when enough moisture was not available in the first one month after transplanting. The main plot was weeded 2 weeks after transplanting. Subsequent weeding was done depending on the intensity of the weeds until the plants were fully established to withstand competition from weeds. In the field, growth data (branching, vertical and horizontal growth) was taken.

2.4.1. Growth parameters

One month after transplanting, four plants for each treatment were selected randomly at the centre core of each subplot and tagged. Boundary plants were avoided. Growth parameters (branching, diameter and height) were monitored and recorded for two years. Direct counts of the number of branches from the leader stem were done. A veneer calliper and a ruler was used to measure diameter and height respectively. As the plants grew big, the veneer calliper and the ruler were replaced with a diameter tape and height rod/suunto hypsometer respectively. Subsequent growth measurements were done on the tagged plants every month for four seasons (two years).

2.5. Data analysis

Charts were generated to depict the levels of branching across the three provenances. Branching data was subjected to Test of Homogeneity of Variances to detect existence of significant differences among the three provenances. Diameter and height data were used to generate horizontal and vertical growth curves respectively for the three provenances.
Growth data was further subjected to Duncan Multiple Range Test (DMRT) to isolate existence of significant differences across the three provenances. Pearson correlation analysis was used to establish existence of relationships across the growth parameters in the three *C. procera* provenances.

### 3. Results

#### 3.1. Production of branches by *C. procera*

It was observed that *C. procera* is generally a multi-stemmed plant (Figure 2). Emergence of branches occurred immediately the plants were transplanted though there were cases where it started at the nursery level especially when the seedlings were affected by cutworms or other injuries that triggered resprouting of new shoots. Subsequent damage in the field by pests, diseases or physical injuries triggered massive branching with some plants taking a bush-like morphology (Figure 2). The bush-like plants were characterized by a minimum of 5 to a maximum of 40 branches.

![Multi-stemmed morphology of *C. procera* at the study site](image)

**Figure 2** Multi-stemmed morphology of *C. procera* at the study site

![Average branching levels of *C. procera* provenances](image)

**Figure 3** Average branching levels of *C. procera* provenances
By the age of 2 years, branching in the three provenances seemed to have levelled off (Figure 3). Tharaka provenance had the highest mean branching of 20.94 followed by Baringo (17.84) then Kibwezi (16.92) as shown in figure 4.4. When the branching data was subjected to Test of Homogeneity of Variances, significant statistical differences (p<0.5) were obtained with Baringo being the least significant (0.043), Kibwezi (0.01) and Tharaka (0.000).

3.2. **Horizontal growth of *C. procera* provenances**

The three provenances showed minor variations in diameter growth in the first nine months of field growth. From the age of 16 months, all the three provenances showed minimal diameter increment (Figure 4) though complete levelling off of horizontal growth curves was not evident by the end of the two year monitoring period. However, mean DBH growth followed a slightly different trend as captured under branching with Tharaka recording the highest (5.74cm), Kibwezi (5.49) and Baringo (5.23). Individual stems showed lack of homogeneity in diameter distribution with Tharaka recording a range of 4.5-7.3cm, Kibwezi (3.8-7.3) and Baringo (3.7-7.4).

![Figure 4 Mean DBH of *C. procera* provenances over two year period](image1)

![Figure 5 *C. procera* stem sizes at the study site](image2)

The observed diameter sizes (Figure 5) at the end of the monitoring period were generally low since a ground check in the course of this study captured a naturally growing *C. procera* plant that had a diameter of 25cm along Enziu river, Mui ward, Mwingi Central, Kitui County. Duncan Multiple Range Test (DMRT) showed no significant statistical differences (p<0.5) in diameter growth for the three provenances. However, when the data was subjected to Pearson correlation analysis, all the three provenances showed strong positive correlations (Pearson, p<0.01) between branching and DBH with Kibwezi recording $r_s = 0.975$, Baringo ($r_s = 0.988$) and Tharaka ($r_s = 0.996$).
3.3. Vertical growth of *C. procera* provenances

In the first one year of growth parameters monitoring, Baringo provenance recorded the highest mean height (Figure 6). However, by the end of the two year monitoring period, Tharaka had highest mean height of 368.24cm, Kibwezi (354.20) and Baringo (339.67) as shown in figure 3.4. Height growth lacked homogeneity typical in plantation crops with each provenance showing a range in height distribution (Tharaka 230-442cm, Kibwezi 233-448.5cm and Baringo 213.5-429cm). At the end of the two year monitoring period, the height growth had not levelled off. Test of homogeneity of variances showed significant statistical differences (p<0.5) with Baringo being the least significant (0.034), Kibwezi (0.022) and Tharaka (0.01). All the three provenances showed a strong positive correlation (Pearson, P<0.01) of branching, DBH and Height with Baringo recording $r_s = 0.975$, Kibwezi ($r_s = 0.988$) and Tharaka ($r_s = 0.996$).

![Figure 6 Height of *C. procera* provenances over a two year period](image)

4. Discussions

4.1. Production of branches

The multiple branches observed in the three provenances is a typical growth characteristic of *C. procera*. The heavy branching is important during the reproduction phase since most of these branches become floral in the reproductive stage. Similar relationship of branching and reproduction phase is documented by [25] who recorded multi-branching characterized by phenophases of leaf flush, flowering and fruiting. Several studies have reported *C. procera* to be multi-stemmed or single stemmed. For instance, [26] in a study on therapeutic potential of *C. procera* described the plant as erect, tall, large and much branched. Elsewhere, [27, 18] stated that *C. procera* occurs as a single or multi-stemmed.

[28] documented that *C. procera* single stemmed or has a few stems. [22] described *C. procera* as a shrub or small tree whose stem is usually simple and rarely branched. Elsewhere, [21], in a study on the demography and size structure of the giant milkweed shrub *C. procera* (Aiton) reported maximum mean branches of 26 compared to the maximum of at least 40 obtained in this study. Probably, the difference can be attributed to the fact that Galal’s branching study was done on *C. procera* growing in the wild while the current study was done in a farm setting. Typically, plants in a farm will always have an added advantage of growing space compared to those growing in the wild.

While the multi-stemmed growth nature of *C. procera* observed in this study may be typical to the species, the study established that damage induced by pests and diseases acted as a stimulus for excessive branching. Sprouting of new branches after loss of apical dominance is a mechanism of recovery from damage of the apex of the leader stem. Damage induced branching as observed in this study is likely to have been hormone mediated. In many instances, such branching is controlled by auxin which is a hormone released after loss of apical dominance to end dormancy of axillary buds and trigger development of new shoots. Thus, the loss of apical dominance due to attack by pests and diseases as observed in some plants in this study is likely to have triggered the release of the hormone which in turn ended dormancy of axillary buds leading to heavy branching. Leveling off of branch production is an indication of plant maturity. Typically, as a plant matures, there is a tendency of reduced active growth hence leveling of increment in branches, height and diameter.

The observed sprouting after loss of apical dominance is in consistence with [29] who documented that loss of apical dominance activates the release of dormant axillary buds to form new branches. It is worthwhile to note that apical
dominance ensures that resources are channeled to the leader stem while activation of dormant buds allows for recovery after damage of the apex of the leader stem. [30] observed that the growth of axillary buds is usually inhibited by the apex-derived auxin but upon loss of apical dominance, dormancy of axillary buds end. Similarly, [31] documented involvement of auxin and cytokinin in shoot flushing. Though branching after damage helps in recovery, [32] cautioned that excessive branching may be costly and hence it is modulated in response to environmental factors. Similarly, [33] reported that excessive branching leads to shading of some of the branches and this decreases the growth especially in shaded branches.

4.2. Horizontal growth of *C. procera* provenances

The lack of differences in diameter growth in the first nine months of field establishment is likely to have been caused by homegeneity in resources allocation in the initial stages of field establishment. However, as the stems grow, competition for resources such as light, water amongst others is expected to start. In presence of competition, the adaptive capability of a provenance to the local conditions will determine its growth. Provenance performance based on environmental conditions has been documented by [16, 6]. Lack of leveling off of diameter size by the end of the two year monitoring period is a clear indication that none of the three provenances had reached full maturity. It is a clear indication that *C. procera* can achieve a higher DBH than the maximum of 7.4cm recorded in some stems. Elsewhere, [18] documented that *C. procera* can have a diameter of 25cm. Hassan's diameter is in consistence with a similar one noted in a *C. procera* stem growing in the wild in the course of this study. In a different study, [34] came up with six diameter classes (>0.5, 0.5–1, 1–1.5, 1.5–2.5, 2.5–3.5, >3.5) in a study on the effects of urban habitat heterogeneity on functional traits plasticity of the invasive species *C. procera*.

The observed slow increment in DBH as the plants approached two years of age can be attributed to reduced meristematic activities at the cambium as the plants age. Similar dependence of cambial activities to tree age has been documented by [35]. Similarly, [36] noted similar relationship of cambial activities and tree age in a mature silver-fir plantation. This study could not authoritatively explain the observed huge diameter ranges for individual stems across the three provenances but provenance genetic heterogeneity was suspected to have been involved. In a typical plantation, individual stems for each provenance should fall in one or two diameter classes and show homogeneity in terms of diameter distributions. However, the influence of soil fettility on the observed differences was ruled out since analysis of soil micro and macro nutrients in the study site didn't capture statistically significant differences.

Though the differences in mean DBH across the three provenances was not high, the low mean DBH in Baringo provenance is likely to have been caused by seed transfer distance. From a geographical location of the seed sources for the three provenances, Baringo was the furthest from the research site followed by Kibwezi then Tharaka. The results of this study support what many researchers have reported that local provenances are better and the further a provenance is moved away from its native homeland, the higher the chances of poor performance. [10] tried to answer the question of how far plant material can be moved from home sites and remain ecologically appropriate in a restoration setting. The study concluded that subjecting a plant material to long distance transfers can result in phenological mismatches between plants and pollinators.

In a study using pine provenances, [18] tried to explore the home-site advantages in seed transfer distance during restoration program. Typically, when a provenance is grown within its native homeland, it will have the advantage of continuing with co-evolutionary processes in the ever changing environment thus giving it the home-site advantage. However, when a provenance is moved away from its homeland, such co-evolutionary processes are interfered with in the new environment. According to [8] concerns exist over the actual scale of adaptation in trees and the relative dangers of incorrect seed source. [37], in a study using 245 white spruce provenances, observed similar differences in provenance performances caused by climatic differences. Similarly, [14], in a study using six *P. tecunumanii* provenances noted similar differences in growth performance.

The results of this study can act as a guideline in future *C. procera* seed collection for maximum growth. Based on DBH performance in this study, Tharaka was the optimum seed source. Typically, an optimum seed source is a geographical locality which can act as a seed source for provenances with the highest growth potential. Based on this concept, it is highly likely that better DBH growth could have been achieved if seeds originated from *C. procera* natural stands near the research site. The importance of seed transfer distance is well documented by [6] who stressed on the need for proper provenance site matching and establishment of seed transfer guidelines.

In a study using 16 provenances, [9] reported local provenance as the threshold at which geographic distance corresponds to statistically significant genetic distance. The seed transfer distance affects latitude and elevation [6]
which in turn determines climatic conditions. [5], in a study on field performance of silver birch provenances in different latitudes, observed that a longer transfer led to decreased yield and the proportion of trees with a stem defect increased.

Increase in the number of branches increases the number of leaves and possibly leads to increased rate of photosynthesis. Increased photosynthesis increases the amount of energy generated and allocated for plant growth. This, probably, explains the observed positive relationship between branching and DBH growth. [19], in a study on effects of urban habitat heterogeneity on functional traits plasticity of C. procera, observed similar relationship between branching and DBH growth. Further, [2], in a modelling study using hardwoods, reported a weak negative correlation between the number of branches and DBH (Pearsons, $r_s = -0.29$).

4.3. Vertical growth of C. procera provenances

The observed relatively low and good performance in mean height by Baringo and Tharaka provenances respectively is likely to have been caused by seed transfer distances. Tharaka provenance seed source being nearer to the research site compared to Baringo probably had a better home-site advantage over Baringo. The seed transfer distance is used in development of seed transfer guidelines in many countries. Such guidelines give local provenances priority and ensure that seeds from a given provenance are not moved over long distances that can lead to pronounced changes in elevation and latitude. It is worth to note that the elevation and latitude are key determinants of climate of a given locality through their influence on temperature.

Influence of latitude on performance of different provenances from different seed sources have been reported by [4, 5, 6, 2] documented the effects of seed origin on the timing of height growth cessation and field performance of Betula pendula from different latitudes. The influence of home-site advantages on provenance performance primarily due to local climatic and environmental conditions have been documented by [11, 12, 13, 14, 15, 10, 8]. Based on this, the current study proposes that C. procera seed collection should strive to minimize the seed transfer distances for optimum performance. Similar assertions on observation of provenance seed transfer distances during restoration activities have been documented by [6, 8, 9].

This study could not authoritatively establish the causes of individual plants variations in stem heights in the even aged C. procera domestication trial plot but probably genetic make-up of the individual provenances played a role. However, micro-site influences primarily due to soil properties was ruled out since analysis of the soil macro and micro nutrients for the different sub-plots did not yield significant statistical differences (P<0.05). Elsewhere, [17] attributed microsite homogeneity to lack of significant differences in provenances performance. Similar microsite homogeneity was recorded in this study.

It is, however, important to note that similar variations in stem heights in even aged stands have been documented. For instance, [38] analyzed individual tree growth pattern in an even aged stand and reported significant variation in individual tree growth pattern in terms of stem height and DBH. Similarly, [39] documented similar height variations in an even aged stand and asserted that by giving priority to allocating resources to height growth over diameter growth, a tree ensures that it maintains its position in the forest canopy, survives and reaches maturity.

The failure of height growth curves for the three C. procera provenances to level off by the end of the two year monitoring period is a clear indication that the plants had not reached their maximum height at maturity and that the obtained maximum height of 4.49m is not the maximum height at maturity. It is evident that given more time, the plant heights will keep on increasing. Therefore, there is need for further monitoring to ascertain the point in time at which the height growth curves will level off at the maximum height and maturity. The mean height obtained in this study are relatively high compared with [21] who reported the highest total mean of individual’s height to be 1.6m. However, the findings are in consistence with [22] who suggested that C. procera is a shrub or small tree that grows up to a maximum of 6m in height.

[18, 40] who asserted that the stem of C. procera occurs as single or multi-stemmed shrub reaching a height of between 2–6m further support the findings. Further, [41] documented that C. procera (Ait.) is an erect spreading shrub reaching a maximum height of 5.5m. [26] described C. procera as a small erect and compact shrub reaching a maximum height of 5.4m. C. procera is a shrub or small tree with a height of between 2–6m [42]. A different species; Calotropis gigantea has been described by [34] as a shrub or a small tree with a height of between 2.5-6m. Generally, from the findings of this study and what other researchers, have reported, it is highly likely that the maximum height of C. procera at maturity is at least 6m.
The reported strong positive correlation in the number of branches, DBH and height in the three provenances can largely be attributed to allometric growth pattern common in many forest shrub and tree species. The findings of this study have been supported by work done by [43, 44] who in a study on the relationships between height-diameter at breast height (DBH), crown diameter-DBH and crown diameter-height of Pinus brutia of Baskonus Mountain, Kahramanmaras, found the strongest relationship to be that of height-DBH relationship. Similarly, [45], in a tree height-diameter relationships comparative study between alpine treeline ecotone and closed forests in Changbai Mountain, Northeastern China, reported similar allometric growth pattern. Elsewhere, [46] observed similar relationship between DBH and height growth for selected tree species and sites in Eastern Texas.

According to [47], the height and diameter relationship does not imply a constant relationship between height and diameter of individual trees. In a study on height-DBH relationship, [48] and Sumida [49] found out that while the trajectories of the most vigorously growing trees had an almost linear height-DBH relationship, trees with a declining DBH growth rate exhibited an asymptotic tendency towards height growth. According to [50], height-DBH relationship is important in describing forest stands and the parameters are key variables in developing volume and biomass models. Further, tree diameter and height are important variables in forest inventory and management and carbon-stock estimation [51].

5. Conclusion
The study concluded that C. procera is a multi-stemmed plant that can reach a height of 4.48m and a diameter of 7.4cm in two years. The nearest seed source (Tharaka provenance) performed significantly better in terms of growth compared to the furthest seed source (Baringo provenance) indicating that seed transfer distances and near home-site advantages played key roles in observed differences.

Recommendations
For good growth, it is recommended that C. procera seeds for raising nursery stock be sourced from the nearest source possible. This will reduce the environmental and climatic effects associated with long distance seed transfers and ensure the species benefits from home-site advantages.

Further studies are required on the key environmental and climatic conditions related to seed transfer distances for C. procera that are responsible for the observed differences in field performance of C. procera provenances. Such information will aid in developing C. procera seed transfer guidelines.

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Disclosure of conflict of interest
The authors declare that there is no conflict of interest in this work.

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