



## Variations in Phenology and Morphology of *Uapaca kirkiana* Müll. Arg. Provenances at Nauko in Liwonde Forest Reserve, Malawi

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### Authors' contributions

This work was carried out in collaboration among all authors. Authors HJ, CRYM, MFC and WS designed the study. Author HJ anchored the field study and gathered the initial data. Authors EM and WS performed the statistical analysis. Authors HJ, CRYM and EM managed the literature searches and produced the initial draft. Author EM addressed subsequent reviewers' comments and suggestions for improvement. All authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** To assess phenological and morphological parameters of *Uapaca kirkiana* Müll. Arg. provenances at sixteen years of age.

**Place and Duration of the Study:** The trial was established in 1997 at Nauko in Liwonde Forest Reserve, Malawi.

**Methodology:** Data collection on phenology and morphology (flowering, fruit production, diameter at breast height (dbh), height, and crown width) of *Uapaca kirkiana* provenances and families was carried out every month from flowering period up to fruiting period (January to May 2014) at sixteen years of age after out planting.

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**Results:** The results show that there were significant ( $P<0.001$ ) variations in flowering sex ratio among the provenances. Phalombe provenance did not deviate significantly from sex ratio equality. The other provenances showed significant male biased sex ratios. There were no significant ( $P>0.05$ ) differences in number of fruits per tree, dbh, height and crown width among the provenances. However, Phalombe had the highest mean number of fruits per tree ( $365\pm158$ ). The mean dbh, height and crown width were:  $10.1\pm0.1$  cm,  $7.7\pm0.06$  m, and  $3.0\pm0.05$  m, respectively. There were no significant correlation between number of fruits and dbh ( $r=0.326$ ;  $P=0.091$ ) and the height ( $r=0.119$ ;  $P=0.547$ ). However, there was significant modest correlation between number of fruits and crown width ( $r=0.454$ ;  $P<0.05$ ). Number of fruits had high heritability value (0.72) followed by height (0.59) and dbh (0.53), while crown width did not appear to be under high genetic control with low heritability value of 0.13.

**Conclusion:** The higher estimated heritability value for number of fruits per tree indicates that phenotypic selection for this trait could be highly efficient. Further studies should investigate heritability and genetic correlation between growth and desirable fruits traits (fruit size, sweetness, pulp ratio, etc) for efficient tree domestication and improvement strategy.

**Keywords:** Sex ratio; number of fruits; diameter at breast height; height; crown width; heritability.

## 1. INTRODUCTION

*Uapaca kirkiana* Müll. Arg., commonly known as “wild loquat” is an indigenous fruit tree found in Miombo woodland of Southern and Eastern Africa, specifically in Angola, Burundi, Democratic Republic of Congo, Malawi, Mozambique, Tanzania, Zambia and Zimbabwe. It belongs to the family of Phyllanthaceae [1]. Under normal condition the tree can attain height of 5-12 m and stem diameter of 5-25 cm. It occurs in areas receiving 500-2000 mm of rainfall per annum and grows in well-drained escarpments, with infertile sand or gravel soils of acidic reaction [2]. The species is dioecious and therefore outcrossing. Insects or specifically bees and wind are the possible pollination vectors. Flowering occurs at the peak of the rainy season and trees can remain in flower for several months. Fruit development commences in the rainy season but extends through the dry season into the next rainy season (5-8 month process) [2,3].

Fruits of *U. kirkiana* are much sought after as a food particularly in times of famine to most forest dependent populations in the region, as they have an extended period of fruit ripening through the dry season. The fruits can be eaten raw, used in making jams, sweetmeats, a refreshing drink and a variety of wines [4]. In Malawi it is highly valued as a source of nutrients and income to the rural communities [5] and in Zambia is of vital socio-economic importance among the rural and urban poor [6].

The natural populations of *U. kirkiana* continue to decline due to deforestation, forest fragmentation and wildfires [7]. Domestication of indigenous

fruit trees is now generally believed to be a conservation measure that will ensure sustainable management and utilization of highly valued fruits [8]. Domestication initiatives by World Agroforestry Center (ICRAF-Southern Africa Programme) for the past two decades have been targeting different indigenous fruit trees including *U. kirkiana* [9]. In addition, ethnobotanical studies within the Miombo ecological zone in Malawi [10] identified *U. kirkiana* as a priority indigenous fruit tree species for conservation and domestication among many indigenous fruit species. Also, in Southern Africa, farmers and stakeholders have recognized the socio-economic value of *U. kirkiana* and identified it as a priority species for domestication [6,11]. In support of this, others have noted that *U. kirkiana* is the commonly preferred indigenous fruit among farmers and consumers [12]. This led to the establishment of an *U. kirkiana* provenance trial at Nauko in Machinga District, Southern Malawi by Forestry Research Institute of Malawi and ICRAF-Southern Africa. The process involved collection and storage of desirable tree germplasm from seven provenances. Currently, the collected germplasm is being evaluated and characterized as fruitful domestication demands identification and use of good genetic germplasm [13].

Knowledge about geographic and genetic variation is a prerequisite in any tree breeding programme [14,15]. So far studies on *U. kirkiana* in the provenance trail have concentrated on growth performance such as height, diameter and crown width [16–18]. Therefore, there is still need to investigate the variation in sex ratio and fruit productivity which may exist among and within provenances. Most economic

characteristics of distinct value in forest trees often have a large amount of individual tree variability, which can be exploited in tree breeding [19]. Provenance and tree to tree variation is responsible for 90% of all genetic variation in forest tree, but the majority of the total phenotypic variation is due to environmental factors [15]. The presence of large variation within and amongst populations may lead to potential for selection and domestication of superior trees into the agroforestry system [20]. Therefore, the objectives of this study were to determine: (1) the variations in fruit productivity among provenances, (2) the correlation between morphometric parameters and fruit productivity, (3) the sex ratio distribution among provenances, (4) heritability values for growth traits and fruit production, and (5) the performance of provenances based on different vegetative growth parameters (diameter, height and crown width) at the age of sixteen years.

## 2. MATERIALS AND METHODS

### 2.1 Study Site

The trial was established at Nauko (35°23 ' S and 15°10 ' E) in Liwonde Forest Reserve located in the southern part of Malawi (Fig. 1). Nauko lies at

an altitude of between 500 m to 1000 m above sea level and experiences a 5-6 month dry season from May to October and a mean annual rainfall of about 840-960 mm. The mean annual temperature range between 18°C – 25°C. Temperature and rainfall during the study period are given in Fig. 2. The soils are ferrallitic latosols with an average pH of 5.2 [21]. Nauko is situated about 320 km south east of Lilongwe the capital.

### 2.2 Experimental Design and Data Collection

The provenance trial was established in 1997. It was laid out as an alpha lattice design with seven provenances, six from Malawi and one bulked from Mozambique and 100 treatments/families for the different provenances [16]. Details of treatments/families per provenance are given in Table 1. There were 20 replications and each treatment or family formed a tree line plot with four trees. A spacing of 2 m within treatments and 4 m between treatments was used. Detailed distribution of the trial, including field management is well explained by Chirwa et al. [16].

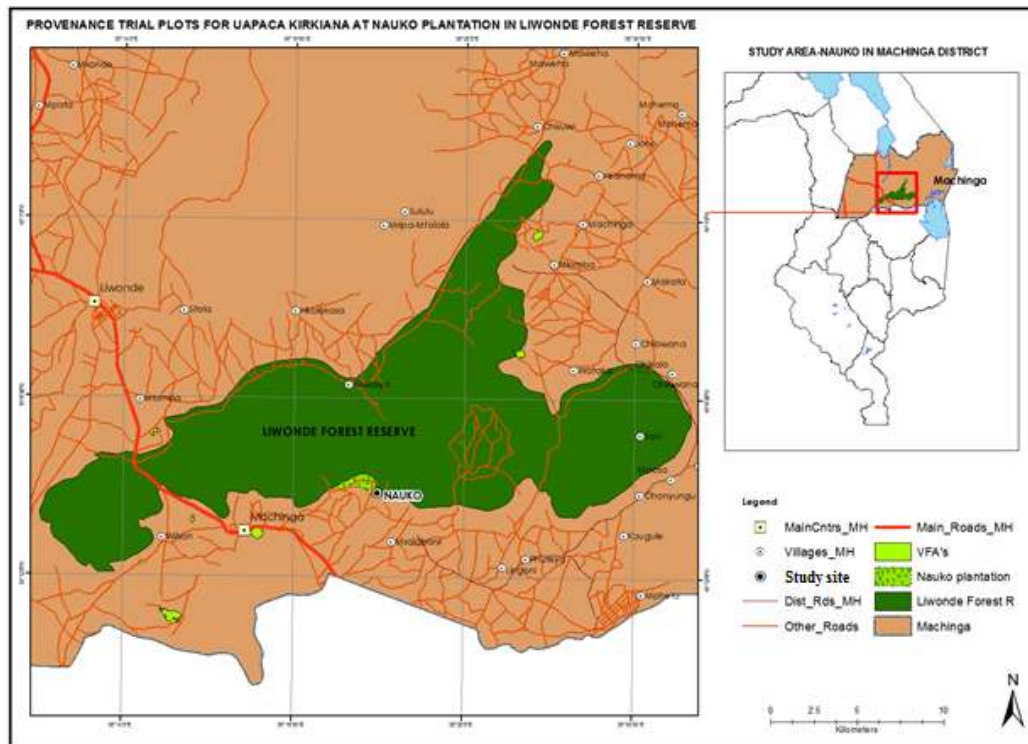


Fig. 1. Location of *Uapaca kirkiana* provenance trial in Liwonde Forest Reserve, Malawi

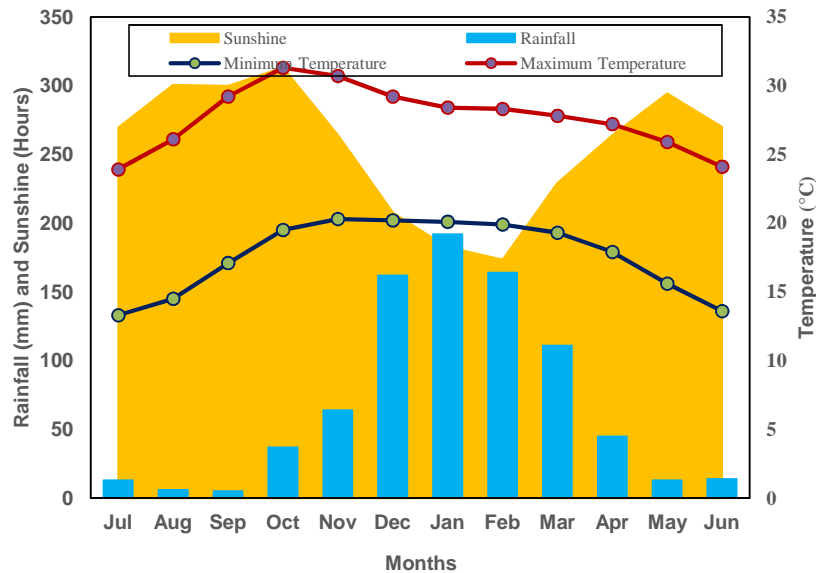


Fig. 2. Weather from July 2013 to June 2014 for the study area

Table 1. *Uapaca kirkiana* provenances and their environmental characteristics

Family code	Provenance	Country	Latitude	Longitude	Altitude (m)	Rainfall (mm)
1-24	Phalombe	Malawi	16°09' S	34°29' E	900	993
25-51	Dedza	Malawi	13°50' S	34°18' E	1623	999
52-64	Thazima	Malawi	10°50, S	33°35' E	1500	1385
65-71	Kasungu	Malawi	12°26' S	35°33' E	1200	870
72-90	Luwawa	Malawi	12°15, S	33°50' E	1200	903
91-96	Litende	Malawi	11°55' S	34°04' E	500	1597
97-100	Bulked	Mozambique	NP	NP	NP	NP

NP= Not presented

### 2.3 Data Collection

Data collection on phenology and morphology (flowering, fruit production, diameter at breast height (dbh), height, and crown width) of *U. kirkiana* provenances and families was carried out every month from flowering period up to fruiting period between January and May 2014. Presence of fruits and retention of withered flowers facilitated sex identification during the assessment. Quantification of fruits was carried out manually by counting number of fruits for each tree with the aid of a tally counter. Height was measured in meters from the base to tip of the tree using a telescopic measuring rod. In case of multi-stemmed individual trees, the tallest leader was measured as the height of the tree. Diameter at breast height (dbh) (1.3 meters from the ground level) was measured to the nearest centimetre using a diameter tape. Where an

individual tree was multi-stemmed after breast height, the mean diameter was calculated and used as a value for statistical analysis. On the other hand, where a tree was multi-stemmed before breast height, then each stem was considered as a separate tree. Where the stem form was irregular at 1.3 m (for example buckles), the dbh was estimated as mean of two measurements taken above and below the irregularity. A 10 m linear tape measure was used to assess crown width measurements. Measurements were made from the south-north direction and the east-west direction. An average of the two measurements was used for statistical analysis.

### 2.4 Statistical Analysis

Data obtained were tested for normality and homogeneity. After the two criteria were met, all

the data were subjected to analysis of variance (ANOVA) using MINITAB for Windows Release 13.30 [22]. Differences between treatment means were separated using Fischer's least significant differences (LSD) at the 0.05 level. The data on dbh, tree height and crown width among provenances was analysed using the following model:

$$Y_{ijkl} = \mu + P_i + F_j + B_k + (PB)_{ik} + (FB)_{jk} + e_{ijkl}$$

Where:

$Y_{ijkl}$  is the random observation on the  $l_{th}$  tree in  $i_{th}$  provenance

$\mu$  is the overall mean

$P_i$ ,  $F_j$  and  $B_k$  are effects of the  $i_{th}$  provenance,  $j_{th}$  family and  $k_{th}$  block, respectively

$(PB)_{ik}$  and  $(FB)_{jk}$  are the effects of provenance-block and family-block interactions, respectively

$e_{ijkl}$  is the random error,  $e_{ijkl} \sim N(0, \sigma_e^2)$ .

The same model was used to analysed data on dbh, tree height, crown width, flowering and fruiting within provenances. The only difference was that the effect of provenance and the provenance-block interaction effect were excluded from the analysis.

A goodness-of-fit test (G-test) in R [23] was used to examine if the sex ratio for each provenance was significantly different from 0.5. Sex ratios were classified as equal when they did not differ significantly from 0.5, male-biased if the proportion of males was significantly greater than 0.5 and female-biased if the proportion of males was significantly less than 0.5. Pearson's correlation coefficient was used to test the correlation between fruit production and vegetative growth parameters (height, diameter at breast height and crown width). Variance components were estimated using SAS Mixed procedure and PROC VARCOMP in SAS software [24], and these were used to estimate individual heritability for all traits. The following formula was used to estimate the narrow sense individual heritability ( $h_i^2$ ):

$$h_i^2 = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_{fb}^2 + \sigma_e^2}$$

Where:

$\sigma_f^2$  is genetic variance due to family within provenances

$\sigma_{fb}^2$  is family block interaction variance

$\sigma_e^2$  is residual or error variance

Standard errors for the heritability were estimated using delta method and PROC IML in SAS software [24].

### 3. RESULTS

#### 3.1 Flowering and Fruiting Trees

Summary of the results of flowering and fruiting trees within provenances of *U. kirkiana* in Nauko Malawi are presented in Fig. 3. The results show that there were significant ( $P < 0.05$ ) differences in number of flowering trees among the provenances. The flowering percentage ranged from 6.8% to 18.8%. Mozambique (18.8%) and Luwawa (18.2%) had the highest number of flowering trees, while Litende had the lowest (6.8%). However, there were no significant ( $P > 0.05$ ) differences in number of fruiting trees among the provenances. The fruiting percentage within provenances ranged from 2.3% (Kasungu) to 5.1% (Phalombe).

#### 3.2 Sex Ratio

Sex was identified in 13.1% of the total trees. There were significant ( $P < 0.001$ ) variations in flowering sex ratio among the provenances (Fig. 4). Only one provenance, Phalombe, did not deviate significantly from equality. The other six provenances showed significant male-biased sex ratios. However, Dedza, Litende and Thazima provenances did not significantly differ in sex ratio. No provenance was significantly female-biased. The overall sex ratio significantly deviated from equality and showed significant male-biased sex ratio. Fig. 5 shows the proportion of females among flowering individuals against proportion of non-flowering individuals.

#### 3.3 Variations in Fruit Productivity

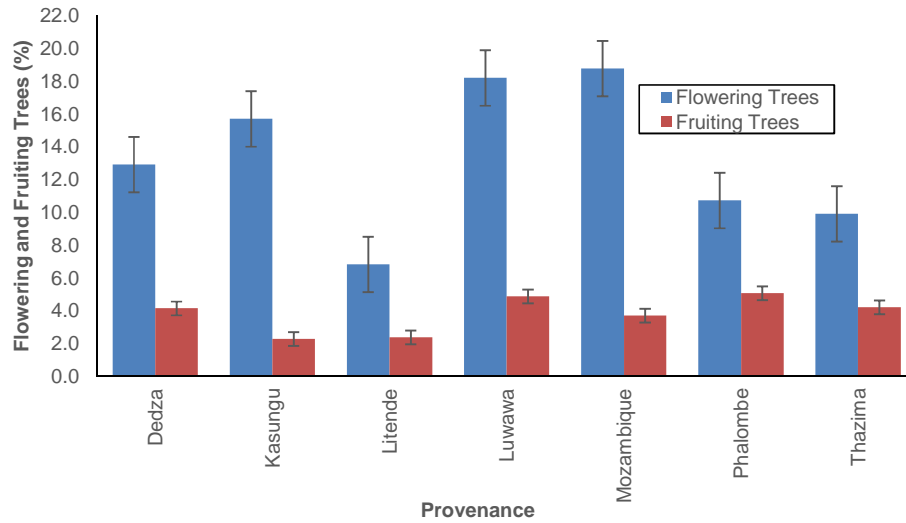
There were no significant ( $P > 0.05$ ) differences in mean number of fruits per tree among provenances (Table 2). However, Phalombe ( $365 \pm 158$ ) and Thazima ( $310 \pm 234$ ) showed the most outstanding fruiting potential. The least fruiting provenance was Mozambique (15).

#### 3.4 Variations in dbh, Height and Crown Width among and within the Provenances

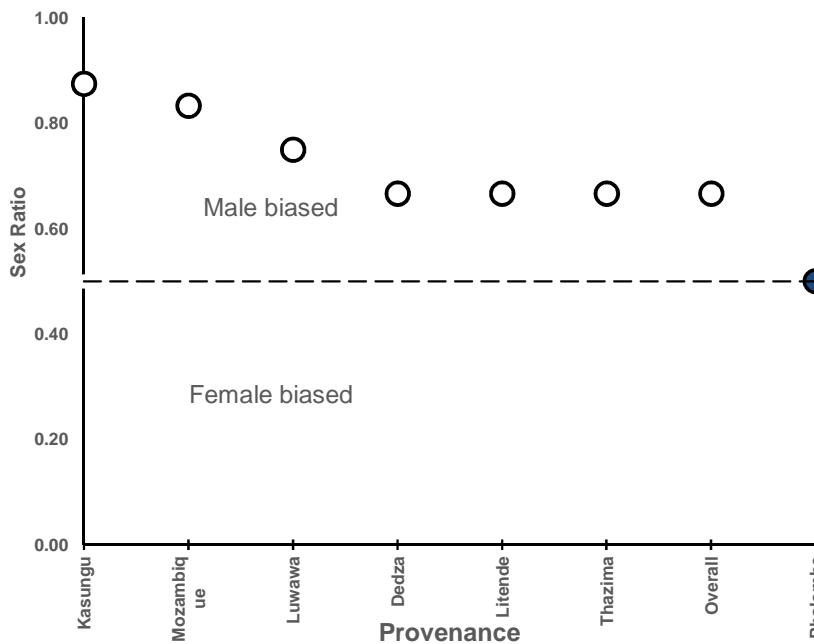
There were no significant ( $P > 0.05$ ) differences on dbh, height and crown width among the provenances (Table 3). However, there were significant differences on dbh, height and crown width among treatments/families in some

provenances. There were significant ( $P<0.001$ ) differences on dbh among families in Phalombe provenance. Tree height were significantly ( $P<0.001$ ) different among families in Luwawa and Phalombe provenances. Dedza also recorded a significant ( $P<0.05$ ) difference on tree

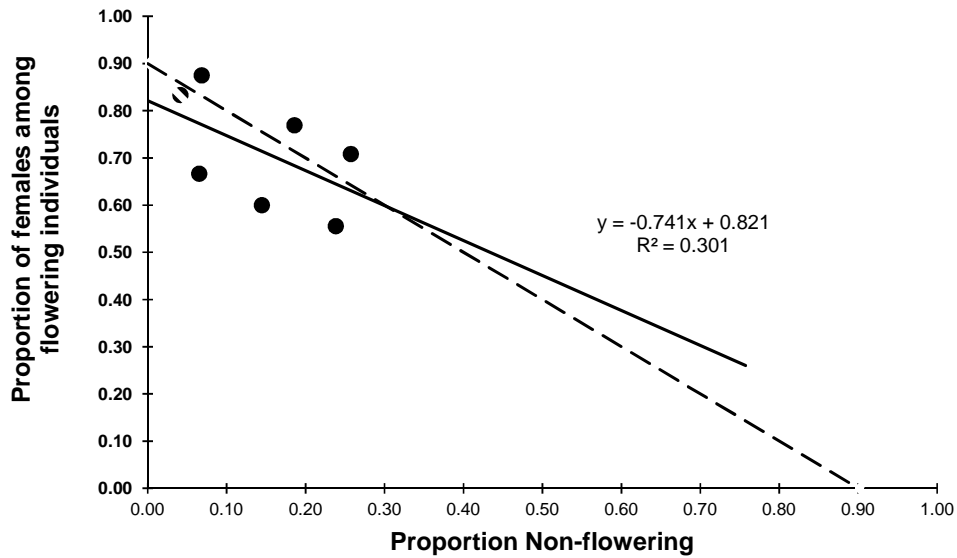
height among families. There were significant ( $P<0.05$ ) differences on crown width among families in all provenances. The mean dbh, height and crown width per families in provenances are shown in Figs. 6, 7 and 8 respectively.



**Fig. 3. Percentage of flowering and fruiting trees within provenances of *U. kirkiana* in Nauko, Malawi**

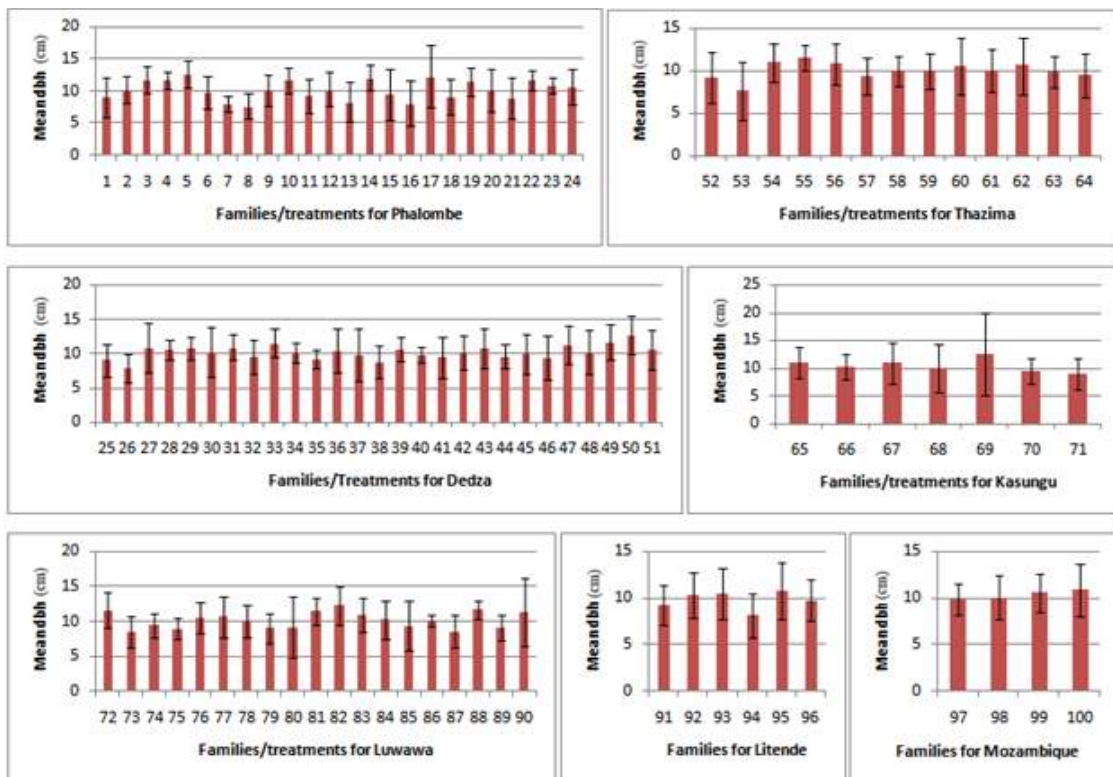


**Fig. 4. Variation in sex ratios among the provenances**  
 Closed circles represent provenances with sex ratios at equality (no significant deviation from 0.5), whereas open circles above and below 0.5 represent provenances with significant male and female bias, respectively



**Fig. 5. Proportion of females among flowering individuals of *Uapaca kirkiana* against proportion of non-flowering individuals**

The solid line is the regression line ( $y=0.821-0.741x$ ,  $r^2=0.301$ ); the dashed line indicates the expected relationship of  $y$  and  $x$  in a population with a 1:1 sex ratio, if all non-flowering plants are female



**Fig. 6. Mean dbh for families within provenances**



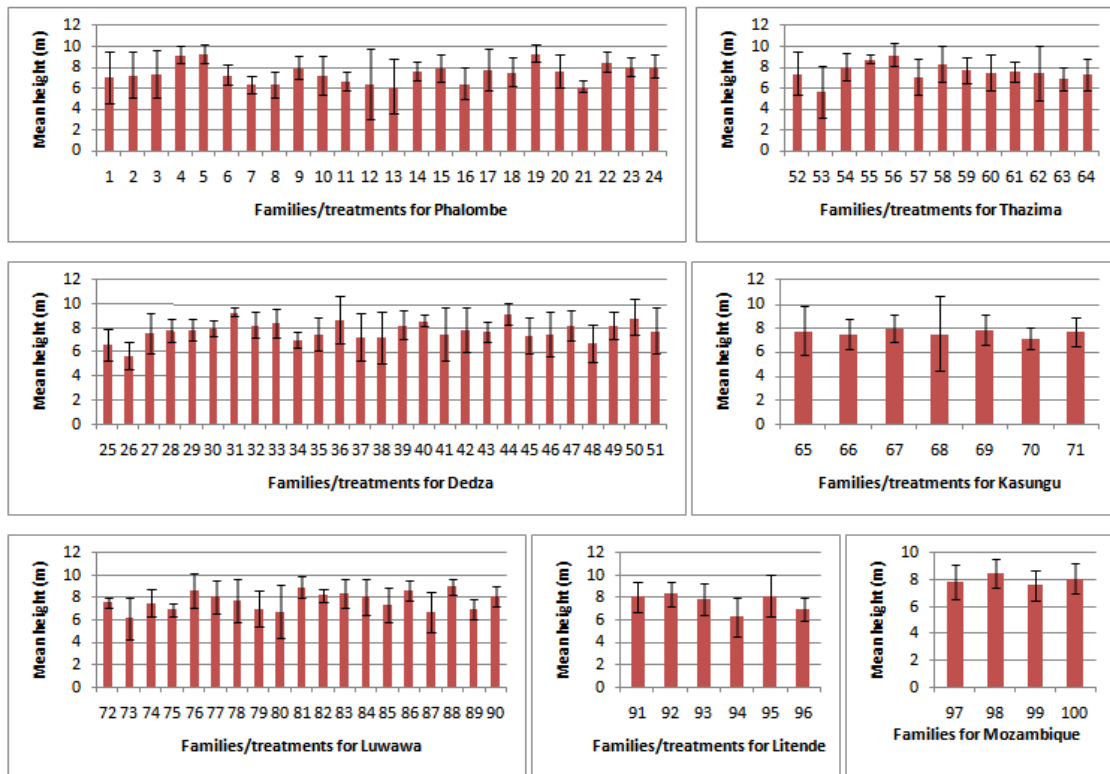


Fig. 7. Mean tree height for families within provenances

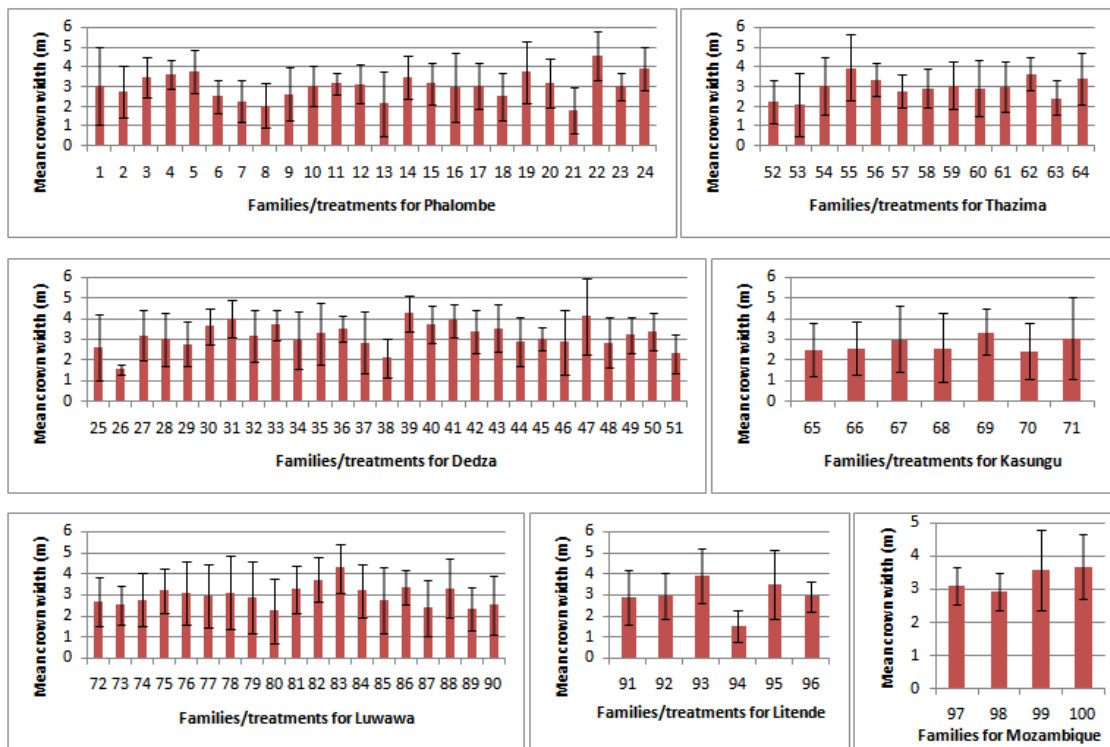


Fig. 8. Mean crown width for families within provenances



### 3.5 Correlation between Growth Parameters and Fruit Productivity

There was significant positive correlation between number of fruits and crown width ( $r=0.454$ ;  $P<0.05$ ). There was a moderate positive correlation between number of fruits and dbh ( $r=0.326$ ) and weak positive correlation between number of fruits and the height ( $r=0.119$ ). However, these correlations were not significant ( $P>0.05$ ) (Table 4).

### 3.6 Heritability Estimates

Variance components, narrow sense individual heritability and the percentage contribution of different factors to the total variation in growth parameters and fruit production are presented in

Table 5. The results show that heritability ranged from 0.13 to 0.72. Number of fruits was most heritable followed by height, dbh and crown width, respectively.

## 4. DISCUSSION

### 4.1 Variations in Number of Flowering and Fruiting Trees and Sex Ratio among Provenances

The results indicated that at 16 years of age about 13.1% of the trees have started flowering and only 4.3% started fruiting (Fig. 3). The results also show that the sex ratio of the population was male biased (Fig. 4). A primary sex ratio of 1:1 is the theoretically expectation in a population [2,4]. However, differences in

**Table 2. Production of number of fruits per tree for fruiting trees per provenance**

Provenance	Mean	Minimum	Maximum	Standard deviation
Dedza	72	12	185	59
Kasungu	106	106	106	0
Litende	42	42	42	0
Luwawa	45	12	88	28
Mozambique	15	15	15	0
Phalombe	365	11	1348	447
Thazima	310	18	1000	468
<i>P-Value</i>	0.738			
CV (%)	205			

**Table 3. Mean dbh, height and crown width among provenances**

Provenance	dbh (cm)	Height (m)	Crown width (m)
Dedza	10.2±0.19	7.8±0.11	3.1±0.09
Kasungu	10.3±0.49	7.6±0.22	2.7±0.20
Litende	9.8±0.38	7.6±0.24	2.9±0.20
Luwawa	10.1±0.21	7.7±0.13	3.0±0.11
Mozambique	10.3±0.38	8.0±0.20	3.3±0.15
Phalombe	10.3±0.22	7.6±0.13	3.1±0.10
Thazima	10.0±0.25	7.6±0.17	3.0±0.12
<i>P-value</i>	0.880	0.792	0.285
Grand mean	10.1±0.10	7.7±0.06	3.0±0.05
CV (%)	26.4	20.7	39.2

*Note: Mean values are followed by the standard error (SE) of the mean*

**Table 4. Correlation matrix for growth parameters and fruit production of *U. kirkiana* at Nauko in Liwonde Forest Reserve, Malawi**

	Diameter at breast height	Height	Crown width
Height	0.491**		
Crown width	0.328 <sup>ns</sup>	0.131 <sup>ns</sup>	
Number of fruits	0.326 <sup>ns</sup>	0.119 <sup>ns</sup>	0.454*

\*\*Significant at  $P=0.01$ , \*significant at  $P=0.05$ , ns=not significant

**Table 5. Variance components, heritability and the percentage contribution of different factors to the total variation in growth parameters and fruit production of *U. kirkiana* at Nauko in Liwonde Forest Reserve, Malawi**

Source of variation	Trait			
	dbh	Height	Crown width	Number of fruits
Family	13.29	14.82	3.21	18.05
Family-Block Interaction	1.20	0.50	0.38	72.21
$\sigma_f^2$	0.0532	0.1068	0.0111	76
$\sigma_{fb}^2$	0.0048	0.0036	0.0013	304
$\sigma_e^2$	0.3423	0.6100	0.3334	41
$h_t^2$	0.53 (0.05)	0.59 (0.05)	0.13 (0.04)	0.72 (0.05)

Note:  $\sigma_f^2$  = genetic variance due to family within provenances;  $\sigma_{fb}^2$  = family block interaction variance;  $\sigma_e^2$  = residual or error variance;  $h_t^2$  = heritability with standard errors in parenthesis

abundance of male and female plants appear to be a consequence of their different reproductive roles, males and females often differ in their resource requirements and may have different responses to the environment. Female plants are subject to greater resource demands that extend over a longer period. Their greater reproductive investment appears to result in slower growth, hence males begin flowering at a slightly younger age than female trees, which could lead to sex ratio biases [25-27].

The bias in the sex ratio in the present study could in part result from differences in flowering frequency between sexes, because most provenances contained non-flowering trees. If one sex flowers less often than the other, this bias should increase as the proportion of the non-flowering individuals increase [25]. We therefore, plotted proportion of females among flowering individuals against the proportion on non-flowering trees (Fig. 5). The regression line has a slight but non-significant negative slope, which indicates that differences in flowering pattern can account for only a small portion of the observed sex ratio bias. This suggests that the effect of different microclimate at the site of planting on the flowering of *U. kirkiana* may greatly contribute to the sex ratio of the species, hence, the need for continuous investigation.

#### 4.2 Variations in Number of Fruits among Provenance

The current results revealed substantial variation in fruit production within and among provenances. Among provenances variation was of high magnitude reaching 689% considering best and lowest mean. Extremely high variation existed at family level for the superior and inferior fruit producers (12254%). The provenance and individual tree variations found in this study can

be used in classical tree breeding and vegetative propagation in order to achieve high genetic gains for the farmers to benefit quickly. Tree breeding may target trees which are producing more fruits in the provenances. Phalombe and Thazima provenances produced more fruits at the moment than the other provenances and selection for superior trees suitable for domestication can be selected from them.

This study recorded a lower number of fruits per tree than those reported by Mwamba [28], exceeding 2000 fruits per tree in Zambia's natural population. Other researchers [11], also reported more than 2000 fruits per tree in Malawi, in natural population. The difference may be attributed to the effect of spacing between trees [2]. Experience from Zambia [28] has shown the fruit yield is adversely affected by stocking densities and canopy closure, where dominant trees carried heavier fruit loads than intermediate-sized or small and suppressed trees. Tree size and its carbohydrate storage capacity is one of the most important factors that determine the number of fruit a tree can nurture to maturity [29]. In this trial 4.3% of the trees fruited and 9.2% were identified as male trees, the rest can be assumed to still be at adolescent stage. This demonstrates that some provenances are early fruit bearers while others are late fruit bearers. This is one of the preliminary reports on fruit production from this trial; therefore there is need for a continued evaluation to investigate if there will be consistency in fruit production by the fruiting trees and to continue monitoring trees which are assumed to still be at adolescent stage. In order to achieve high fruit productivity silviculture practices, such as thinning, should be initiated. Care should be taken in order not to remove female trees which are few in number.

### 4.3 Variations in dbh, Height and Crown Width among and within the Provenances

The results revealed that there were no significant ( $P>0.05$ ) differences on dbh, height and crown width among provenances. This is an indication that no provenance is superior to the other in terms of dbh, height, and crown width. In contrast, earlier studies supported significant differences in growth parameters [17]. The current study shows that the earlier differences in dbh, height, and crown width among provenances has phased out at the age of sixteen years.

### 4.4 Correlation between Growth Parameters and Fruit Productivity

The results from this study show a significant modest correlation between crown width and fruit productivity and no significant correlation between fruit productivity and dbh, and also between fruit productivity and tree height. Crown width is possibly a good parameter for choosing an ideotype for fruit improvement. Fruit trees that have a thick, healthy, and spread-out crown produce more fruits than fruit trees with a small, sparse and unhealthy canopy [4]. The current result is in disagreement with what other researchers [30] found on *Sclerocarya birrea*, another fruit tree in Namibia, Southern Africa. For *S. birrea*, there was a good correlation between crown size and fruit yield ( $r=0.67$ ,  $P<0.05$ ) and between tree girth and fruit yield ( $r=0.59$ ,  $P<0.05$ ). This implies that the current results on *U. kirkiana* cannot be used at the moment in indirect selection of fruit production potential based on growth parameters because this may end up with large error margins. Hence, continuous investigation is needed.

### 4.5 Heritability Estimates

Heritability estimates obtained in the present study ranged from low, moderate to high. Number of fruits had high heritability value (0.72), while height (0.59) and dbh (0.53) had moderate heritability values and crown width (0.13) had low heritability value. The very high value of heritability for number of fruits per tree indicate that genetic factors are more important than environmental factors for this trait. On the other hand, the low value of heritability for crown width indicates it is primarily controlled by environmental factors. The higher estimated heritability value for number of fruits per tree

indicates that phenotypic selection for this trait could be highly efficient. These results agree with those reported by other researchers [7].

The present study was based on one growing season and, therefore, may have uncertainty in variations among provenances because of growth with seasonal fluctuation. Hence, continuing research for several consecutive years is needed to fully establish the genetic and phenotypic characteristics of *U. kirkiana*. This would help in understanding the variations which occurred due to seasonal variations in each growing season period [31]. Heritability and genetic correlation between growth and desirable fruits traits (fruit size, sweetness, pulp ratio, etc) should be further investigated for efficient tree domestication and improvement strategy.

## 5. CONCLUSION

The present study shows that there is substantial variation on sex ratio occurring among the provenances. Phalombe provenance, did not deviate significantly from sex ratio equality, while the other six provenances showed significant male-biased sex ratios. However, Dedza, Litende and Thazima provenances did not significantly differ in sex ratio. There were no significant differences on number of fruits per tree, dbh, height and crown width among the provenances. However, Phalombe showed a high potential on mean number of fruits per tree. There were no significant correlation between number of fruits and dbh, and between number of fruits and height. However, there was significant modest correlation between number of fruits and crown width. Number of fruits was most heritable followed by height, dbh and crown width. The higher estimated heritability value for number of fruits per tree indicates that phenotypic selection for this trait could be highly efficient. Further investigation should be carried out on heritability and genetic correlation between growth and desirable fruits traits (fruit size, sweetness, pulp ratio, etc) for efficient tree domestication and improvement strategy.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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