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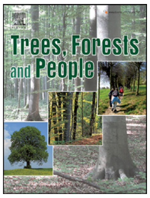

Diversity in fruit and seed morphology of wooden banana (*Entandrophragma bussei* Harms ex Engl.) populations in Tanzania

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ABSTRACT

Entandrophragma bussei (wooden banana) is a high value indigenous multipurpose tree species prioritized for domestication in Tanzania. However, we lack adequate information on genetic and phenotypic variation to support domestication processes, while utilization pressure on the remaining stocks and deforestation are intensified. The study evaluated the diversity in fruit and seed morphology of three wild populations of *E. bussei* found in three agroecological zones of Tanzania. Data on fruit (length, width, weight and number of seeds per fruit) and seed (length, width and weight) traits were evaluated. To detect differences in means among the populations, one-way Analysis of Variance (ANOVA) was performed. There were variations in fruit and seed morphological traits among the studied populations. Ruaha population had significantly higher fruit length (19.31 ± 0.1 cm), width (7.71 ± 0.12 cm) and number of seeds per fruit (22 ± 0.48) than Kigwe (15.65 ± 0.14 cm, 4.85 ± 0.17 cm, 20 ± 0.45), and Tarangire (16.84 ± 0.1 cm, 5.40 ± 0.12 cm, 20 ± 0.37) populations. Ruaha (62.46 ± 1.37 g) and Tarangire (60.71 ± 1.12 g) had significantly heavier fruits than Kigwe (56.53 ± 1.28 g). Kigwe population had significantly higher seed width (1.80 ± 0.01 cm) and weight (0.83 ± 0.01 g) than Ruaha (1.75 ± 0.01 cm, 0.75 ± 0.01 g) and Tarangire (1.65 ± 0.01 cm, 0.77 ± 0.01 g) populations. Among the populations Tarangire had higher seed length (9.60 ± 0.06 cm) than the rest. Overall, there is a considerable fruit and seed morphological diversity among the *E. bussei* populations offering opportunities for selection of domestication cultivars and gene pool conservation.

1. Introduction

Entandrophragma bussei Harms ex Engl. or wooden banana is a priority multipurpose indigenous tree species that belongs to the family Meliaceae. While other species of the genus *Entandrophragma* are distributed in several countries across Africa, *E. bussei* is endemic and found in arid, semi arid and areas around Lake Victoria in Tanzania (Msanga, 1998). Almost all parts of the tree are used for numerous purposes, including as a source of wood for fuel, construction, furniture and handicrafts in Tanzania (Makonda and Batiho 2018). The roots, leaves and barks are used by both rural and urban dwellers to cure different human ailments including diarrhoea, anaemia, laxatives, worms, hypertension, charm, body pains, asthma, urinary infection, trypanosomiasis, chest and abdominal problems, and general ailments (Dery et al., 1999; Rao *et al.*, 2004; Monela *et al.*, 2005). The extracts of various parts contain secondary metabolites and other lead compounds (Nibret *et al.*, 2010; Mouthé-Happi *et al.*, 2018), which explain the traditional uses in folk medicine. Infact Traditional healers may consider using the tree to fight against corona virus infections caused by the recent COVID 19 outbreaks. As there has not so far been any effective vaccination and treatment, *E. bussei* may potentially continue to be used by many communities to treat many ailments caused by COVID 19 infections. The tree was selected by local communities as one of the priority species among 10 for domestication programmes of the World Agroforestry Centre in the Western Tanzania (Dery and Otsyina, 2000). There are generally few studies on the genus *Entandophragma* and available literature has focused mainly on the distribution (Msanga, 1998; Yakusu *et al.*, 2018), seed dispersal (Medjibe and Hall, 2002), germination (Msanga, 1998; Hall *et al.*, 2003), medicinal value (Dery and Otsyina, 2000; Mouthé-Happi *et al.*, 2018) and wood properties (Lemmens, 2008; Yakusu *et al.*, 2018).

Despite the benefits offered by the *E. bussei*, the species is facing mounting pressure from over exploitation due to booming trade in indigenous medicinal trees and as alternative hardwood timber species to mahogany (Makonda and Batiho, 2018). Moreover, the extent of deforestation in the country, estimated at 469,420 ha per annum (URT, 2017), implies that the natural habitats for the species are dwindling. In addition, lack of comprehensive programmes to restore and conserve native tree species has exacerbated the negative effects on the remaining pop-

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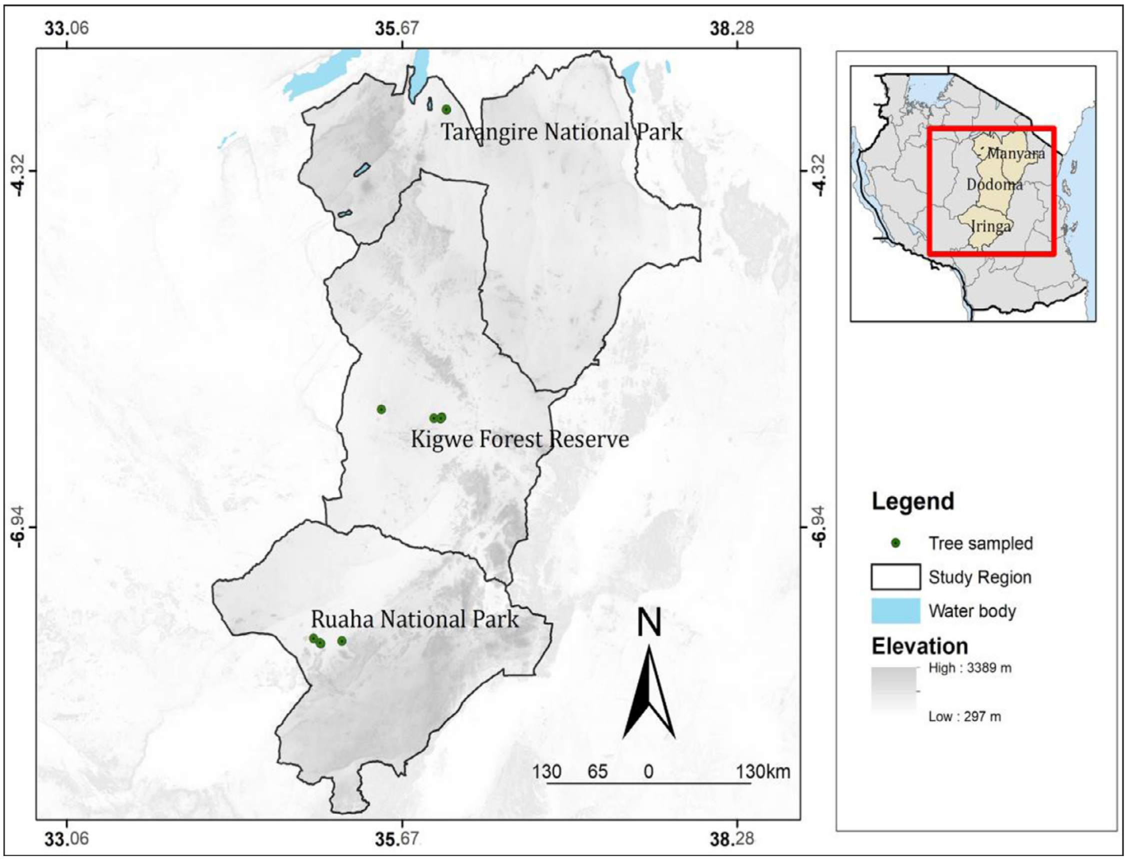

Fig. 1. Locations of three sampled *Entandrophragma bussei* populations in Tanzania.

ulations of *E. bussei* in the wild (Yakusu *et al.*, 2018). This trend calls for appropriate mitigation strategies to increase *E. bussei* conservation and production through domestication to avoid risks of extinction. However, domestication of indigenous tree species is limited by inadequate knowledge that could have supported establishment and maintenance of high value trees like *E. bussei* in different land uses. Most of the available literature has focused on socio-economic importance of *E. bussei* (Dery, 1999; Nibret *et al.*, 2010), while information on phenotypic and genetic structures remains unknown. A recent shift of attention of reforestation programmes towards indigenous trees and the need to prevent their extinction has made it clear that scientific efforts should be directed to the understanding of the silviculture of native plants, including *E. bussei* (Makonda and Batiho, 2018). *E. Bussei* is propagated only through seeds (Msanga, 1998). To our understanding this is the first study on morphological diversity in fruit and seed traits of *E. bussei* populations. Information of phenotypic variation is a prerequisite for the domestication and improvement of *E. bussei* trees from the wild (Zobel and Talbert, 1984; Dawson *et al.*, 2009). This study assesses the variability in fruit and seed morphological traits of three populations of *E. bussei* occurring in Arid, Semi-arid and Southern Highland agroecological zones of Tanzania (TNBS, 2015). It is hoped that findings of this study will contribute to the development of future domestication and tree improvement programmes for *E. bussei* in Tanzania.

2. Materials and methods

2.1. Study sites

The study was carried out in Kigwe Forest Reserve (Dodoma region), Tarangire National Park (Manyara region) and Ruaha National Park (Iringa region) (Fig. 1) from June to August 2020 in Tanzania. These sites were selected based on known major occurrences of *E. bussei* from published information and herbarium specimens.

The three sites are all protected, although national parks have relatively higher level of protection compared to forest reserves. No human activities are allowed except nature-based tourism and research in national parks, while in forest reserves activities such as fallen deadwood collection, beekeeping and collection of top soil for nursery establishment are permitted. Ruaha site has undulating plains to dissected hills and mountains with flat treeless savannahs, miombo woodlands (from which fruits were collected), dry bush lands, swamps and riverine forests. It experiences unimodal (December-March), reliable and local rain shadows. Kigwe site is characterized by undulating plains with rocky hills and low scarps with wooded grassland, bush land with thickets and scattered *Adansonia digitata* trees. Kigwe experience unimodal (December-March) and unreliable rainfall. Illegal activities such as timber harvesting and encroachment for farming are present in the reserve. Tarangire site is characterized by low ridges of gneiss and pre-Cambrian rocks and valleys. The landscape is covered with *Acacia-Combretum* woodland, grassland and *A. digitata* stands. It experiences bimodal rainfall pattern, with long (March-May) and short very unreliable rains (November - December). Other details for the three study sites are presented in Table 1.

2.2. Sampling

Before actual sampling, reconnaissance field survey was done to major known sites with occurrence of *E. bussei* populations in which a total of six regions (Iringa, Dodoma, Manyara, Kilimanjaro, Singida and Tabora) were visited. Finally, three regions with at least 20 remaining individual trees each were chosen for fruit and seed studies. At each site, 15 trees were randomly selected and tagged. Diameter at breast height (DBH, cm), height of a tree (m), and geographical location of each num-

Table 1
Characteristics of fruit collection sites for three populations of *Entandrophragma bussei* in Tanzania.

Populationname	Agroecological zone	Latitude(°S)	Longitude(°E)	Altitude(m)	Annualrainfall(mm)	Temperature(°C)	Soil characteristics
Ruaha	Southern Highlands	7°75´ – 7°79´	34°98´ – 35°20´	915 – 974	800 – 1400	15 – 25	Poorly drained black cotton and leached red-brown sand soils
Kigwe	Semi-arid	6°08´ – 6°15´	35° 51´ – 35°98´	979 – 1098	500 – 800	15 – 32	Well drained soils with low fertility, less regular soil pattern including brown sand, clay sand and stony soils
Tarangire	Arid	3°87´ – 3°88´	36°01´ – 36°02´	1167 – 1222	500 – 600	16 – 27	Well drained, medium textured, stony and Montmorillonite black cotton soils on valley bottoms

Table 2
Characteristics of *Entandrophragma bussei* plus trees sampled for fruit collection in Tanzania.

Population	Characteristics	Unit	Mean	SE	CV	Min	Max
Ruaha	DBH	cm	79.4	9.45	0.27	54	108
	Height	m	16	0.4	0.06	15	17
Kigwe	DBH	cm	117	8.89	0.17	100	150
	Height	m	16.2	0.58	0.58	15	18
Tarangire	DBH	cm	124.8	8.63	0.15	104	145
	Height	m	18	1.12	0.14	15	20

SE: Standard error, CV: Coefficient of variation, Min: minimum, Max: maximum.

bered tree were recorded and used in the selection of plus trees. Height and DBH of plus trees were measured by hypsometer and diameter tape, respectively (Table 2).

Finally, five plus trees were sampled for fruit collection at each site. A tree was considered a plus tree if it had no pests and diseases symptoms, had straight and circular stem of large diameter, possessed fine horizontal branches, found between other neighbouring trees and if it had large quantities of fruits compared to others.

2.3. Data collection

From each plus tree, 20 ripe fruits without any damage or malformation were collected making 100 fruits per population and 300 fruits for the three populations. Fruits and later seeds were aggregated and treated on a population basis. Fruits were harvested by climbing the tree and picking by long handle secateurs randomly. Falling fruits were caught on a tarpaulin laid on the ground to avoid cracking of the fruit shell. The collected fruits were packed and labelled appropriately including information on collector, date of collection, identity and location, and transported to the Directorate of Tree Seed Production (DTSP) Laboratory in Morogoro for further measurements of morphological traits.

Fruits were measured: length, width, weight and number of seeds per fruit. Fresh fruits were weighed to the nearest gram with a digital balance, whereas length and width were measured using electronic digital calliper with a precision of 0.01 mm. Fruits from each population were then left to dry under the house shade for 14 days to allow natural opening of the capsules. On drying, fruits were split from the tip towards the base and released seeds with a wing attached to the central column. Seeds were extracted by shaking the capsules and cleaned by hand to remove debris. A sample of 400 clean seeds was randomly selected using a soil divider and systematically marked with number 1 to 400 for each population. Thus, a total of 1200 seeds were sampled for the three populations for measurements of length, width and weight.

2.4. Data analysis

Before analysis, all fruit (length, width, weight and the number of seeds per fruit) and seed (length, width and weight) morphological traits were examined for standard assumptions of parametric test using standard diagnostic plots in package ggplot2 (Wickham, 2016) and Shapiro-Wilk's Test. Fruit and seed lengths and width, and number of seed per fruit were not normally distributed at $\alpha = 0.05$. Thus, these traits were log transformed to reduce skewness and to improve homoscedasticity. Analysis of Variance (ANOVA) was generated using the general linear model. Variations of fruit and seed morphological traits among the three populations were analyzed using One-way ANOVA. Wherever significant differences were found, means were compared with Tukey's Honestly Significant Difference (Tukey's HSD) post hoc test. Pearson Product Moment Correlations (r) were used to evaluate the relationship between different fruit and seed morphological traits. All data analyses were treated on population basis in R version 4.0.3 (R Development Core Team, 2020).

3. Results

3.1. Fruit variation among populations

Fruit length differed significantly among the populations (Table 3), with the Ruaha population having the longest fruit and Kigwe having the shortest. Fruit width was significantly different among populations, with Ruaha population having the largest fruit width and Kigwe having the smallest. There was a significant variation in fruit weight among populations, with Ruaha and Tarangire having the heavier fruits than Kigwe. Number of seeds per fruit differed significantly among populations, with Ruaha having the largest number of seeds than Kigwe and Tarangire. The coefficient of variation in fruit length, fruit width, fruit weight and number of seeds per fruit ranged from 11 to 31% (Table 3). The overall means for fruit length, width, weight and number of seeds were 17.3 cm, 6 cm, 59.9 g and 21.

There were significant correlations between fruit and seed morphological traits in the present study (Table 4). Fruit length was positively correlated with fruit width, fruit weight and number of seeds per fruit. Fruit width related positively with fruit weight and negatively with seed weight. There was a positive correlation between fruit weight and number of seeds and fruit weight and seed weight. The number of seeds correlated negatively with seed length. Seed length related positively with seed weight. Seed weight correlated negatively with fruit length and positively with seed width (Table 4).

3.2. Seed variation among populations

Seed length varied significantly among the populations (Table 5), with Tarangire having longer seeds than Ruaha and Kigwe. There was

Table 3
Variation in fruit morphological traits among the three populations of *Entandrophragma bussei* in Tanzania.

Population	Fruit length (cm)(means ± SE)	Fruit width (cm)(mean ± SE)	Fruit weight (g)(mean ± SE)	Number of seeds/fruits(mean ± SE)
Ruaha	19.31 ± 0.1b	7.71 ± 0.12b	62.46 ± 1.37b	22 ± 0.48b
Kigwe	15.65 ± 0.14a	4.86 ± 0.17a	56.53 ± 1.28a	20 ± 0.45a
Tarangire	16.84 ± 0.10c	5.40 ± 0.12c	60.71 ± 1.12b	20 ± 0.37a
F-value	250.9	117	5.97	5.26
P-value	<0.001	<0.001	<0.01	<0.01
SE	0.11	0.11	0.73	0.26
CV	0.11	0.31	0.21	0.22

Means followed by a common letter in the same column are not significantly different at $p < 0.01$, Tukey's HSD Test. Means are followed by the Standard Error (SE) of the mean. CV= Coefficient of variation.

Table 4
Pearson correlation coefficients for fruit and seed morphological traits of *Entandrophragma bussei* populations in Tanzania

Trait (Unit)	Fruit length	Fruit width	Fruit weight	No. of seeds per fruit	Seed length	Seed width
Fruit width (cm)	0.77***					
Fruit weight (g)	0.50***	0.61***				
No. of seeds per fruit	0.79***	0.08	0.54***			
Seed length (cm)	0.02	-0.03	-0.03	-0.25**		
Seed width (cm)	0.04	0.03	0.02	0.07	0.08	
Seed weight (g)	-0.18*	-0.17*	0.27**	-0.09	0.32**	0.40**

* Significant at $p < 0.05$.
** Significant at $p < 0.01$.
*** Significant at $p < 0.001$.

Table 5
Variation in seed morphological traits among the three populations of *Entandrophragma bussei* in Tanzania.

Population	Seed length(cm)(mean ± SE)	Seed width(cm)(mean ± SE)	Seed weight (g)(mean ± SE)
Ruaha	8.43 ± 0.05a	1.75 ± 0.01b	0.75 ± 0.01b
Kigwe	8.48 ± 0.05a	1.80 ± 0.01a	0.83 ± 0.01a
Tarangire	9.60 ± 0.06b	1.65 ± 0.01c	0.77 ± 0.01b
F-value	163.7	56.91	33.49
P-value	<0.001	<0.001	<0.001
SE	0.03	0.01	0.004
CV	0.13	0.12	0.19

Means followed by a common letter in the same column are not significantly different at $p < 0.001$, Tukey's HSD Test. Means are followed by the Standard Error (SE) of the mean. CV= Coefficient of variation.

no significant difference in seed length between Ruaha and Kigwe populations. Seed width varied significantly among the populations, with seed width largest in Kigwe and Tarangire having the smallest. Seed weight was significantly different among the populations, with Kigwe having heavier seeds than Tarangire and Ruaha. There was no significant difference between seed weight of Tarangire and Ruaha populations. The coefficient of variation in seed weight, seed width and seed weight ranged from 12 to 19% (Table 5). The overall means for seed length, width and weight were 8.8 cm, 1.7 cm and 0.8 g.

4. Discussion

4.1. Variation in fruit size, weight and number of seeds

There was high variation of fruit and seed morphological traits among populations in the three agroecological zones. The study sites are found in different agroecological zones characterized by different altitude, temperature, rainfall and soils. *E. bussei* fruits from Ruaha with relatively wetter climate were longest, widest, heaviest and with largest number of seeds in fruits as compared to the fruits from Kigwe and Tarangire which have semi-arid and arid climates, respectively. Differences in fruit and seed morphological traits have been widely reported in other socially and economically important trees from other parts of Africa. For example, it has been reported that fruits of *Adansonia digitata* (baobab) which happens to be distributed widely and often associated with *E. bussei* in Tanzania, have significantly higher mean weight in the wetter than the driest areas of Mali (De Smedt *et al.*, 2011). Fruits of *A. digitata* have also been reported to be longer, wider and heavier in humid climate as compared to areas close to semi-arid regions in Kenya (Omondi *et al.*, 2019). In this study, it is therefore possible that the observed variations in fruit traits are a result of differences in climate and environmental conditions such as precipitation, and soil characteristics. It has also been reported that genetic diversity among the selected plus trees contributes to overall differences in fruit morphological traits among populations (Ngulube *et al.,* 1997). Thus, differences in genetic makeup among the chosen plus trees might likely have contributed to the observed fruit morphological trait diversity in the studied population in addition to climate and environmental conditions.

A positive and significant relationship was found between fruit weight, length, width and the number of seeds in this study (Table 4). Similar results were reported in other important multipurpose tree species in East (Omondi *et al.*, 2019), West (Parkouda *et al.*, 2012, van den Bilcke *et al.*, 2014) and Southern (Sanchez *et al.*, 2011) Africa. The significant correlations between morphological fruit traits indicate that one trait can be used to predict other traits especially in field where time and finances are often constraints (Dicko *et al.*, 2019). The mean fruit length of *E. bussei* from Ruaha (19.31 cm) and Tarangire (16.84 cm) were longer than that of *E. caudatum* (15 cm) from Southern Africa (Yakusu *et al.*, 2018) and *E. angolense* (15.44 cm) from equatorial climate areas of West Africa (Dike and Aguguom, 2010). Also, fruit length reported from Kigwe (15.65 cm) is comparable to that of Shinyanga with semi-arid climate (15 cm) in Tanzania (Derry *et al.*, 1999). Fruit width of *E. bussei* from Tarangire (5.50 cm) is similar to that of *E. angolense* (5.52 cm) from equatorial climate areas of West Africa (Dike and Aguguom, 2010).

4.2. Variation in seed size and weight

Seed morphological traits varied too among the study populations. Kigwe population had the largest seed width and heaviest seeds than Ruaha and Tarangire populations. Tarangire population had the longest seeds than Ruaha and Kigwe populations. The observed variations in seed length, width and weight among populations are likely to be caused by climate (Fredrick *et al.*, 2015), soils (Ngulube *et al.*, 1997) and genetic (Abasse *et al.*, 2011) differences as also explained for fruits. The studied populations had overall longest seeds (average of 8.84 cm) than *E. caudatum* (2.5 cm) from Southern Africa (Yakusu *et al.*, 2018). Similarly, overall seed weight was higher in the studied populations (average of 0.78 g) than those reported for *E. angolense* (0.48 g) and *E. cyndricum* (0.41 g) from tropical climate areas of West Africa (Dike and Aguguom, 2010). Fruit and seed morphological traits correlated well suggesting that those factors affecting fruits also affects seeds proportionally. The sink strength of fruit depends upon the number and size of the seed they contain as seed constitute priority sink with higher capacity to mobilize translocases compared to other fruit parts (Cannell, 1985). It is important to consider strong seed traits such as seed weight during tree selection for domestication and tree improvement. Heavier seeds are important because the heavier the seed, the higher the nutrients reserved in it and the higher the chance in supporting seed germination and early seedling growth and development (Mwase *et al.*, 2006; Mkwezalamba *et al.*, 2015; Martinez-Gonzalez et al., 2020). Kigwe population displayed higher seed weight than the other populations suggesting that moderate water stress has influenced reservation of much substrate in seeds to enhance germination, seedling growth and survival (Loha *et al.*, 2006; Munthali *et al.*, 2012; Martinez-Gonzalez *et al.*, 2020). Since seed weight correlated highly with seed length it is also likely that high moisture stress is circumvented by the length of seeds in Tarangire population.

5. Conclusions

This study evaluated diversity in fruit and seed morphological traits among the three wild populations of *E. bussei*. We detected significant variation in fruit and seed traits that could offer opportunities for phenotypic selection of superior trees with improved productivity for domestication. The observed variation could be due to environmental and climate differences among the study sites and maternal genetic variations from the sampled trees. High correlation of fruit and seed traits show that field sampling can be conducted based on few key traits rather than the whole range of traits during plus tree selection in domestication processes. Given the magnitude of variation reported in this study, selection of any studied morphological trait for *E. bussei* tree improvement would be effective at population level.

Declaration of Competing Interest

None.

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