GENETIC DIVERSITY WITHIN AND AMONG SOUTHERN AFRICAN PROVENANCES OF *UAPACA KIRKIANA MÜELL. ÅRG* USING MORPHOLOGICAL AND AFLP MARKERS

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ABSTRACT

Domestication of Uapaca kirkiana Müell. Arg is a high priority for improving rural livelihoods of smallholder farmers in southern and eastern Africa. Domestication efforts require knowledge of ecological adaptive traits and intra-specific variation. Morphological traits and amplified fragment length polymorphic (AFLP) markers were used to assess genetic variation in twelve provenances of *U. kirkiana* collected from five countries in southern and eastern Africa. Assessment of tree morphological traits showed significant differences ($p < 0.05$) between provenances where Zimbabwean and Zambian provenances grew much faster than those from Tanzania and Malawi (except Phalombe). Mean Nei's (H) genetic diversity of AFLP showed high diversity within the provenances H= 0.181 to 0.321 with mean of 0.256. An analysis of molecular variance (AMOVA) showed that most genetic variation (90.8 %) resided within provenances, while only 8.2 % was variation among provenances. There was no geographical pattern of variation in growth and morphological traits among the seed sources. Chipata provenance from Zambia was the most diverse while Mapanzure from Zimbabwe was the least diverse but more superior in height growth and earliest in fruiting. The pattern of genetic diversity in *U. kirkiana* indicates existence of genetic drift and high gene flow between provenances suggesting that regional collections and conservation strategies should consider differences by focussing on the main range of the species.

Keywords: AFLP - Conservation- Domestication- Genetic diversity- Provenance- *Uapaca kirkiana*- *UPGMA*

INTRODUCTION

Uaaca kirkiana Müell. Årg (family Euphorbiaceae) known as African wild loquat is a tree indigenous to the miombo woodlands of eastern, central and southern Africa (Ngulube *et al.* 1995). The tree can grow in the range of 11 to 13 m with a juvenile phase of 9 to 10 years for planting materials derived from sexual propagation, while vegetatively propagated materials can take 2 to 4 years to fruit (Mhango 2000; Akinnifesi *et al.* 2006). The seed is dispersed by humans and animals such as birds, bats, monkeys and rodents (Ngulube *et al.* 1995). Several studies have reported the utilization of *U. kirkiana* fresh and processed fruits as important source of nutrients and income to local communities (Saka and Msonthi 1994; Saka *et al.*, 2008; Ham *et al.* 2008). The fruit pulp is mixed with maize or millet meal, the seeds being discarded and the mixture is eaten uncooked. The ripe fruit pulp, broken up and stored in water, is sometimes left to ferment, making a sweet wine (Mashingaidze *et al.* 1991)

A regional-wide survey undertaken to determine needs and preference of farmers and various users in southern Africa had identified *U. kirkiana* as a priority indigenous fruit tree for utilization, conservation and domestication (Maghembe *et al.* 1998; Akinnifesi *et al.* 2004a). Although *U. kirkiana* is not commercially cultivated, but efforts

are in progress to domesticate and commercialise it in southern Africa (Akinnifesi *et al.* 2006), and it forms part of a global initiative to promote indigenous fruit trees in agroforestry for community livelihood benefits (Leakey *et al.* 2005). As part of a larger domestication program by the World Agroforestry Centre (ICRAF) and its partners in southern Africa, a extensive rangewide germplasm collection was done from five countries, namely: Malawi, Mozambique, Tanzania, Zambia and Zimbabwe—as the first step in a domestication strategy (Akinnifesi *et al.* 2004, 2008). These collections provided materials for assessments, conservation and future utilization in live gene banks, as regional multi-locational provenance trials were established from the collections in 1996 in Malawi, Zambia and Zimbabwe (Akinnifesi *et al.* 2004b). Conservation of genetic diversity is a fundamental goal of conservation biology and knowledge of the extent and structure of genetic variation in provenances of *U. kirkiana* is essential not only for understanding processes of evolution, but also for development of appropriate and efficient strategies for collection, conservation and domestication of superior populations. Studies on genetic diversity of provenances from southern Africa will be crucial for determining strategies to ensure that greatest amount of genetic variation is captured for germplasm collection, conservation and domestication.

Several molecular markers are available for studying genetic diversity in plants. Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.* 1995) based on the polymerase chain reaction (PCR) are spread all over the genomes and are hypervariable. Use of AFLP markers is advantageous in generation of large number of markers spanning the whole genome without prior knowledge of the sequence of the genome. Amplified fragment length polymorphism has been used successfully in plant population genetic studies of tree species (Muluvi *et al.* 1999; Kremer *et al.* 2005; Cao *et al.* 2006). So far, only one study has been reported on their application in population studies of *U. kirkiana* (Mwase *et al.* 2006b) and another study used random amplified polymorphic DNA (RAPDs) (Agufa 2002). Understanding the morphological and phenological variations among trees, fruits, seeds and seedlings (Mwamba 1995; Ngulube *et al.*, 1997; Mwase *et al.* 2006a) from populations derived from different geographical areas are also central to the study of genetic diversity. Although diverse geographical origin per se cannot be considered a genetic parameter, traits like growth rate have the advantage of direct relevance. In order to detect genetically differentiated lineages that may impact on domestication and tree improvement programmes, combining both morphological and genetic criteria parameters are crucial for measuring genetic diversity.

The present study is part of a wider project, which seeks to explore opportunities for the selection, domestication and cultivation of *U. kirkiana* in southern Africa (Akinnifesi *et al.* 2008a). The domestication of *U. kirkiana*, as most other indigenous fruit trees in Africa is a new undertaking and involves a continuous process of improvement that has to be justifies by the benefits accrued to the users—collectors, marketers and consumers. Domesticating trees from wild genepools imposes some responsibilities on scientists to develop an understanding of the potential of the species; to ensure that domestication proceeds wisely, efficiently and within constraints imposed by the Convention on Biodiversity, and to maintain and protect the diversity of genetic resource base (Leakey *et al.* 2004) The aim of this study is to assess the level of genetic variation within and among *U. kirkiana* provenances of southern African based on molecular and morphological tree attributes, and to recommend appropriate germplasm collection and conservation strategies.

MATERIALS AND METHODS

Seed sources

Range-wide collections of *U. kirkiana* provenances were made from five countries of southern Africa, namely: Malawi, Mozambique, Tanzania, Zambia and Zimbabwe during November 1995 and January 1996 (Akinnifesi *et al.* 2004). Seeds were randomly collected from 15 - 20 superior trees of twenty four provenances and exchanged among the five countries making sixteen provenances and twelve of which are part of this study (Table 1). The four countries represent part of the geographical range of natural distribution of *U. kirkiana* in southern Africa region defined by geographical and political boundaries (Figure 1). The seedlings were established in multi-location trials with 12 to 16 provenances in each of the four countries, i.e. at Chipata in Zambia, Iringa in Tanzania, Domboshawa in Zimbabwe and Makoka in

Malawi (Akinnifesi *et al.* 2004, 2006). The experimental design generally employed across countries was randomized blocks with 20 replications. Each treatment consisted of a line-plot of four-trees planted at 2m spacing within row and 4m between rows. The data reported in this study is based on the data collected from the provenance established at Makoka Agricultural Research Station in Zomba, in southern Malawi (15^0 30' S and 35^0 15' E; altitude 1029 m above sea level). The rainfall is unimodal, with most of the rain occurring from November to April. The total annual rainfall ranges from 560 to 1600mm, with a 30-year mean of 1024 mm. The soils at the site are classified as Ferric Lixisol (FAO/UNESCO). The soil texture is 46% sand, 46% clay and 8% silt. The clay content in the site increases with soil depth, but major chemical characteristics are relatively constant to > 1 m depth (Akinnifesi *et al.* 2008).

Measurements of growth and morphological traits

Tree height and stem diameter (diameter at breast height and root collar diameter) were measured every year from 1998 to 2006. Height was measured to the nearest centimetre using a telescopic measuring pole and diameter to the nearest 0.1 cm using callipers. In addition each tree was scored subjectively for morphological traits: stem straightness; branch angle; length of primary branches, crown depth and fruiting. Details of the scoring procedures are provided in Table 2.

Plant material and DNA Extraction

A total of 96 individual *U. kirkiana* trees representing twelve provenances were used for leaf sampling at Makoka Agricultural Research Station. Young leaves about 500 mg were harvested and preserved in silica gel in 50 ml tubes. Total genomic DNA extraction followed the protocol of Hodgetts *et al.* (2001) and Patterson *et al.* (1993), with minor modifications (Mwase *et al.* 2006b). DNA quality was checked by ethidium bromide staining on a 1% agarose gel and concentration was estimated by visual assessment relative to 1 kb DNA (New England Biolabs) ladder of different known concentrations.

Amplified fragment length polymorphic markers assays

The AFLP analysis was performed as described by Vos *et al.* (1995), but with minor modifications (Mwase *et al.* 2006b). AFLP products were separated on a 6% (w/v) denaturing polyacrylimide gel and visualised with silver nitrate. The gels were rinsed in ultra pure water three times and dried at room temperature in fume hood for seven hours. AFLP fragments were scored as present (1) or absent (0) for each primer pair between 50 and 500 bp and variations in band presence were recorded as polymorphism.

Table 1: Source of germplasm of *U. kirkiana* established at ICRAF - Makoka, Zomba in Malawi

Code	*Provenance*	*Country of origin*	*Latitude (^{0}S)*	*Longitude (^{0}E)*	*Elevation (m)*	*Rainfall (mm)*
1	Phalombe	Malawi	16º09'	34º29'	*1260*	*1000*
2	Chimaliro	Malawi	12º15'	33º50'	*1338*	*1200*
3	Litende	Malawi	11º49'	34º10'	*1120*	*1500*
4	Chipata	Zambia	13º40'	32º40'	*1050*	*980*
5	Choma	Zambia	16º51'	27º04'	*1200*	*800*
6	Serenje	Zambia	13º03'	30º37'	*1559*	*1200*
7	Mpwapwa	Tanzania	6º05'	35º46'	*1330*	*550*
8	Mbeya Kyela	Tanzania	9º45'	33º30'	*>2000*	*1500*
9	Iringa	Tanzania	7º50'	35º46'	*1540*	*1100*
10	Nyamukwarara	Zimbabwe	18º40'	32º55'	*1800*	*1450*
11	Murelwa	Zimbabwe	17º40'	31º50'	*1470*	*800*
12	Mapanzure	Zimbabwe	20º20'	31º00'	*1100*	*650*

Table 2 : List of growth and morphological traits of Uapaca kirkiana and their description

Trait	Unit	Explanation
Quantitative traits		
Total Height	cm	measured from root collar to tip
Bole height	cm	measured from the root collar to first living branch of the tree crown
Diameter at breast height	cm	measured at 1.3 m above ground
Root collar diameter	cm	measured at 3 cm above the soil surface
Bark thickness	cm	measured using bark gauge at 3 cm above soil surface
Crown depth	cm	Measured from starting point of branches to stem tip
Branch length	cm	measured from stem to branch tip for primary branches
Branching habit		
Branch angle	1-2	1= upright, $< 60^0$; 2= horizontal $>60^0$
Branchlet length	cm	measured from stem to branch tip for two randomly selected branches per whorl
Qualitative traits		
Stem straightness	1-5	1 = not vertical > 2 bends 2 = roughly vertical >2 bends 3 = roughly vertical, 1-2 bends 4 = roughly vertical and straight 5 = completely vertical and straight
Survival	Percent	Based on 4 tree –row plot
Reproduction		
Fruiting	1-2	1 = yes ; presence of fruits 2 = no: not fruiting

Statistical analysis

Analysis of variance for growth traits was performed across provenances and separately within each country of origin using the general linear model in MINITAB 14.0. Variance across provenances was analyzed according to a linear model with the following sources of variation: provenance and replication as random terms and country as a fixed term. The standardized traits mean values of the morphological data were used to perform cluster analysis using NTSYS pc 2.1 (Rohlf 2000) and a dendrogram was constructed using the unweighted pair group method of arithmetic average (UPGMA). Genetic distances between provenances were calculated with the squared Euclidean distance (Sneath and Sokal 1973), AFLP binary matrices were analysed by Arlequin 2.0 (Schneider *et al.* 2000) for calculating average difference between all genotypes in the population (Tajima 1993) and average gene diversity levels (H) over loci. Hierarchical structuring of genetic variation within and among provenances and pair wise FST distances was determined by an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992). A matrix of corrected average pairwise differences between all pairs of provenances generated by Arlequin was used for constructing dendrograms using UPGMA in NTSYS-pc 2.1 (Rohlf 2000). Simple matching coefficients of similarity (Sneath and Sokal, 1973) were calculated for all pair wise comparisons among provenances. Matrices of Euclidean dissimilarity coefficients based on morphological and AFLP data sets were tested for correlation using the Mantel test. A matrix of geographical distances among provenances was obtained (*http://.www.jan.ucc.nau.edu/-cvm/latlongdist.html*) and compared with the corresponding simple matching similarity coefficients to investigate possible association between geographical and genetic distance (MXCOMP in Mantel test in NTSYS). Data from the AFLP matrix and morphological traits were subjected to principal component analysis (PCA) (Esbensen *et al.* 2007) in Unscrambler 9.7 to investigate further relationships among provenances.

RESULTS

Morphological traits of provenances

The mean absolute values of the 14 growth and morphological characters used in the

characterization are presented in Table 3. Differences within the southern Africa region were highly significant ($p < 0.001$) for diameter at breast height (dbh) and significant ($p \leq 0.05$) for total height, bark thickness, branch length, crown depth and stem straightness. Provenance differences within countries were not significant different for dbh, bole height and root collar diameter. There were significant differences ($p \leq 0.05$) between the provenances for the traits crown spread, leaf length and breadth, fruiting and plant survival indicating a wide range of diversity across the provenances.

There were significant differences in plant height, with provenances from Zimbabwe- Murelwa, Mapanzure and Nyamukwalala attaining the highest height followed by Choma from Zambia while Chimaliro and Litende from Malawi were the shortest (Table 4).

Genetic differentiation

Analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) revealed that 91.0 % of the genetic variation is among individuals within provenances. Although variation among provenances is also highly significant ($p < 0.001$), it contributed to only 7.7% of the total molecular variance (Table 5). Partitioning of the overall variance into two hierarchical levels using AMOVA revealed that differences between countries and altitudes accounted for only 1.6 and 1.0 % respectively. A random permutation indicated that the proportions of variance attributed to countries and provenances were significant ($p = 0.0381$, $p = 0.000$ respectively), while the variance attributed to altitude was not significant ($p = 0.107$). Overall, levels of genetic diversity within provenances did not vary greatly. The average genetic differentiation (FST) for the provenances was 0.0922 with individual population differentiation ranging from 0.002 to 0.259. Nei's measure of gene diversity (H) ranged from $H = 0.181$ to $H = 0.322$ with Mapanzure from Zimbabwe being least diverse while Chipata from Zambia was the most diverse.

Table 3 : Summarised results for 16 growgh and morphological traits of *Uapaca kirkiana* showing mean values and significant different levels

Trait	Region	Countries within Region	Provenance within country
Total height (cm)	322.95 ± 8.58*	322.26 ± 9. 380*	348.6 ±16.2***
Bole height (cm)	79.66± 3.59*	79.66 ± 3.59***	89.93 ± 6.13 ns
Dbh (cm)	5.10± 0.097***	5.024 ± 0.152***	5.157 ± 0.238 ns
Root collar diameter (cm)	6.47 ± 0.133ns	6.75 ± 0.210 ***	8.057 ± 0.336 ns
Bark thickness (cm)	0.87 ± 0.023*	0.7850 ± 0.037*	0.807± 0.0376 ns
Crown spread (cm)	2.18 ± 0.049ns	2.15 ± 0.083 ns	2.2525 ± 0.093 ns
Crown depth (cm)	2.63 ± 0.061*	2.65 ± 0.126 ns	2.657 ± 0.126 ns
Branch length (cm)	52.26 ± 1.38 *	54.43 ± 2.13*	56.45 ± 1.83 ns
Branchlet length (cm)	21.63 ± 0.962 ns	21.30± 0.82 ns	21.88 ± 1.68 ns
Branch angle (degrees)	51.40 ± 0.902 ns	55.13 ± 2.30*	44.93 ± 4.39 ns
Leaf length (cm)	13.763 ± 0.385 ns	13.11 ± 0.568 ns	13.85 ± 1.11 ns
Leaf breadth (cm)	5.86 ± 0.262 ns	4.80 ±0.312 ns	5.04 ± 0.57 ns
Height crown ratio (m)	1.36 ± 0.062*	1.30 ± 0.025 ns	1.3125 ±0.0304 ns
Fruiting (1; 0)	1.97 ± 0.012 ns	0.075 ± 0.042 ns	0.0750 ± 0.0422 ns
Stem survival (0;1)	2.275 ± 0.085 ns	2.40 ± 0.195ns	2.475 ± 0.199 ns
Stem straightness (0-5)	3.45 ± 0.232*	2.27 ± 0.103 ns	3.395± 0.124 ns

Table 4 : Mean values for 8 growth and morphological traits of a 10-year old Uapaca krkia kirkiana provenance trial at Makoka, Malawi

Provenance	Ht	Dbh	Rcd	Brth	Crd	Brcl	Brca	Strt
Phalombe	359.2	5.25	8.10	0.820	2.730	58.90	55.0	3.440
	(29.2)	(0.48)	(0.712)	(0.0512)	(0.279)	(3.36)	(0.762)	(0.277)
Chimaliro	316.1	5.07	7.87	0.870	2.76	65.40	62.10	3.630
	(30.2)	(0.524)	(0.804)	(0.092)	(0.204)	(2.99)	(2.59)	(0.21)
Litende	356.6	4.58	7.41	0.770	2.66	50.50	54.10	3.33
	(43.1)	(0.740)	(0.586)	(0.088)	(0.319)	(3.68)	(2.66)	(0.297)
Chipata	345.2	3.69	8.21	0.80	2.29	47.40	57.30	3.71
	(21.0)	(0.384)	(0.727)	(0.081)	(0.147)	(3.09)	(6.91)	(1.72)
Choma	408.9	5.53	8.83	0.91	2.88	47.60	57.30	3.71
	(16.1)	(0.337)	(0.664)	(0.070)	(0.214)	(2.75)	(2.18)	(0.51)
Serenje	314.2	4.11	6.590	0.680	2.350	42.90	49.30	3.050
	(31.9)	(0.3200	(0.501)	(0.055)	(0.210)	(3.52)	(3.29)	(0.283)
Iringa	366.0	5.72	8.69	0.860	2.36	45.90	49.30	3.050
	(23.4)	(0.465)	(0.724)	(0.056)	(0.196)	(2.40)	(6.94)	(0.203)
Mbeya	348.4	5.200	8.490	1.23	2.950	55.50	60.70	3.61
	(26.6)	(0.490)	(0.654)	(0.220)	(0.211)	(0.274)	(1.92)	(0.205)
Mpwapwa	353.7	5.590	9.36	1.0	2.93	54.80	51.60	3.7
	(17.8)	(0.368)	(0.439)	(0.050)	(0.169)	(2.96)	(6.89)	(0.132)
Nyamukwalala	359.3	5.20	8.12	0.860	2.35	47.70	49.60	3.04
	(33.9)	(0.524)	(0.821)	(0.0095)	(0.295)	(3.55)	(6.25)	(0.385)
Mapanzure*	436.4	7.41	10.88	0.850	2.740	51.30	53.10	3.42
	(37.3)	(0.639)	(0.766)	(0.075)	(0.362)	(3.40)	(6.26)	(0.389)
Murelwa	440.3	7.18	11.08	0.990	2.760	56.10	59.60	3.480
	(29.0)	(0.5100	(0.666)	(0.060)	(0.303)	(3.63)	(3.10)	(0.389)

Ht = total tree height; dbh = diameter at breast height; rcd = root collar diameter; brth = bark thickness; crdp = crown depth; brcl = branch length; brca = branch angle; strt = straightness; * Fruiting observed. The numbers in parentheses are standard errors of the means of traits.

Table 5: AMOVA for 96 individuals of *U. kirkiana* from twelve provenances in southern Africa using 90 AFLP markers

Source of variation	*d.f.*	SS	MS	Variance component	Percent variation	F_{ST}	*p* value
Among countries	3	73.271	24.42	0.2029	1.58	0.092	<0.038
Among provenances within countries	8	156.417	19.55	0.983	7.65		<0.000
Within provenances across 4 countries	84	981.250	11.68	11.681	90.78		<0.000
Among 3 altitude groups	2	48.352	24.18	0.129	1.00	0.090	> 0.107
Among provenances within altitude groups	9	181.335	20.15	1.058	8.22		<0.000
Within provenances across 3 altitude groups	84	981.250	11.68	11.681	90.78		<0.000
Among provenances across countries	11	229.688	20.880	1.149	8.96	0.089	<0.000
Within provenances across countries	84	981.250	11.681	11.681	91.04		<0.000

Using AFLP data, UPGMA separates the twelve provenances into five clusters that did not consistently reflect their geographic origin (Figure 3). The main cluster contains seven

provenances from Phalombe, Chimaliro and Litende (Malawi), Chipata and Serenje (Zambia), and Mbeyakyela and Iringa (Tanzania). The provenance Mpwapwa from Tanzanian clusters with Nyamukwalala from Zimbabwe in the second cluster whilst the third, fourth and fifth clusters consists of a single provenance each from Zambia and Zimbabwe. The provenance Mapanzure with the lowest genetic diversity is clearly separated from the rest of the provenances.

In general, the topology of Figure 3 showed some similarity to that provided by morphological traits, however Mantel test show that the correlation between the two dissimilarity matrices was not significant ($r = 0.52$, $p = 0.09$). Moreover, the Mantel test revealed no significant correlations between AFLP based differentiation and geographic distance between pairs of populations ($r = 0.26$, $p = 0.558$).

Table 6: Genetic variation in twelve provenances of *U. kirkiana* based on AFLP data

Provenance	PPL	Average difference	Nei's gene diversity (H)
Phalombe	58.8	22.42	0.249 ± 0.140
Chimaliro	58.8	22.71	0.252 ± 0.141
Litende	53.3	20.03	0.247 ± 0.139
Chipata	77.7	28.92	0.321 ± 0.179
Choma	74.4	26.14	0.290 ± 0.162
Serenje	47.8	18.00	0.222 ± 0.125
Mpwapwa	52.2	18.57	0.235 ± 0.133
Mbeya Kyela	56.7	23.50	0.261 ± 0.146
Iringa	61.0	22.89	0.254 ± 0.143
Nyamukwarara	65.6	23.89	0.277 ± 0.156
Murelwa	67.8	25.17	0.280 ± 0.156
Mapanzure	38.9	15.60	0.181 ± 0.103

PPL, percentage polymorphic loci; the average difference is mean number of pair wise differences between individuals in the provenances

Principal component analysis

There was considerable overlapping between individuals from different provenances and countries as discerned from the principal component analysis performed on the AFLP matrix. Three clusters were observed (Figure 4) and the first two principal components accounted for only 22% of the variation, corresponding to 16% for PC1 and 6% for PC2. Principal component 2 separated Mapanzure from the rest of the populations. The results clearly distinguished Mapanzure from the rest of the provenances and seem to be significantly distinct from others while most provenances from Malawi, Tanzania and Zambia form one group cluster I. Inspection of the loading plots showed that certain alleles were missing in Mapanzure, however it also had two markers that distinguished it from the rest of the provenances. Most of the provenances seem to cluster independently from their geographical origin. This well exemplified in the case of Nyamukwalala, Chimaliro and Iringa provenances that are distantly located exhibited close PCA clustering distribution. The biplot of PCA (figure not shown) obtained with the two marker sets showed morphological traits specifically total height discriminating some populations by their geographic origin.

DISCUSSION

The *U. kirkiana* regional provenances grown under the same condition showed varied growth and morphological traits. The geographic source of germplasm for domestication is an important consideration, which in practical application is based on the degree of local adaptation among provenances as well as the cost and availability of material from different areas. Use of local provenance as a germplasm source may ensure better adaptation to the local conditions and reduces transport costs. It is not so clear, how much of the variation can be allotted to differing environmental conditions in the source areas, or if

the tested populations represent different ecotypes, as is also possible within an area. The pattern of genetic variation along a geographical distribution is confounded by specific local environmental conditions.

Apparently, apart from Chimaliro from Malawi, most provenances collected from Zimbabwe (Mapanzure and Murelwa), Zambia (Choma), Tanzania (Iringa) and Malawi (Phalombe) have good adaptive growth traits at the test site in Malawi. Akinnifesi *et al.* (2004b) reported that provenances are more adapted to climatic conditions at their origin, but the pattern and degree of local adaptation varies among species. In this trial, provenance ranking in terms of tree height and diameter was not always consistent with results conducted in Zambia where Chipata and Choma provenances had greatest growth (Akinnifesi *et al.* 2004b). The good performance of these provenances could be attributed to similarity of the climatic factors, especially rainfall and elevation between the source of the seed and test site. The lack of consistency in the results at the two sites indicates the likely presence of significant provenance and environment interactions, further analysis of results of the regional provenance trials is required to determine the extent of genetic variation in the selection of superior provenances that can perform consistently in diverse climatic conditions.

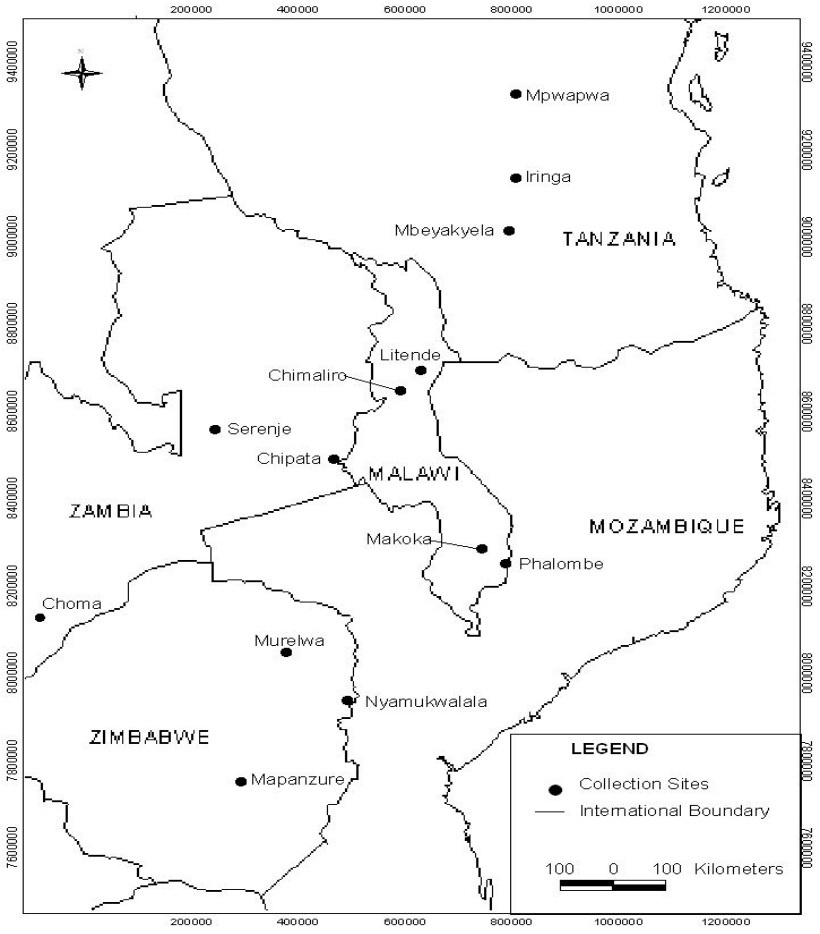

Figure 1: Map of southern African countries showing location of twelve provenances as sources of germplasm planted at ICRAF-Makoka, Zomba in Malawi

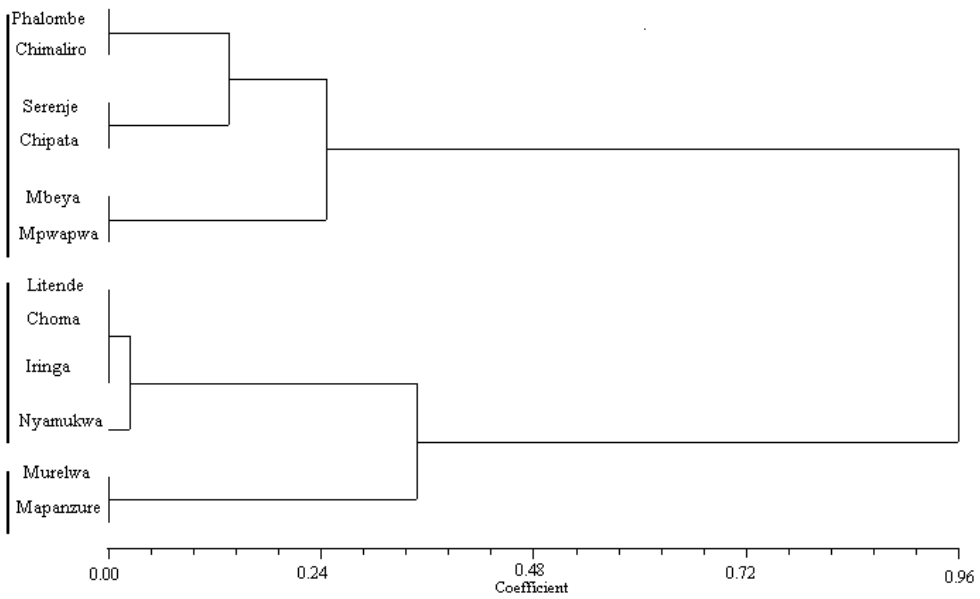

Figure 2: Dendrogram of twelve southern African *U. kirkiana* provenances derived from UPGMA from dissimilarity matrix of morphological data

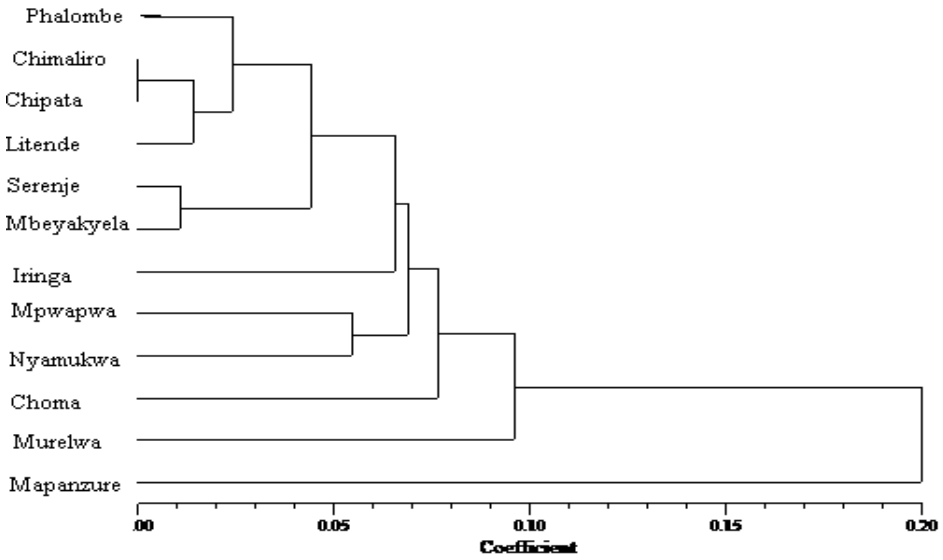

Figure 3: Dendrogram of twelve provenances using 90 polymorphic AFLP markers based on UPGMA of corrected average pairwise differences

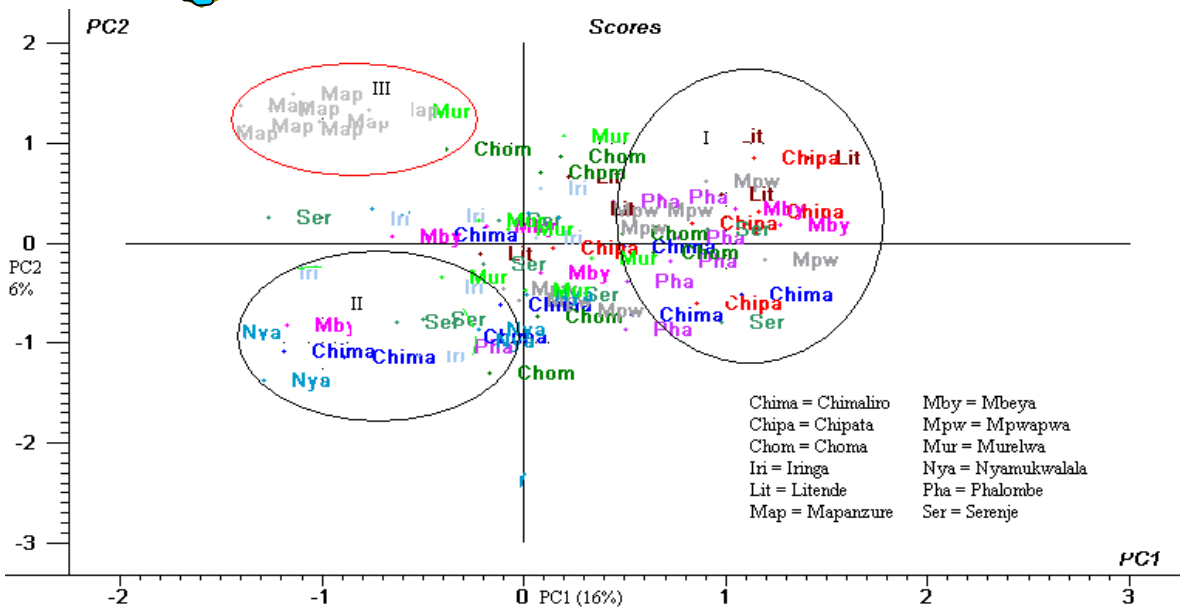

Figure 4: PCA plot of twelve provenances of *U. kirkiana* based on AFLP polymorphic markers

In the present study, we have used AFLPs to study genetic diversity within and between twelve provenances of *U. kirkiana*. Significant levels of variation and moderate levels of differentiation have been reported in the same species using AFLPs for natural populations collected from Malawi (Mwase *et al.* 2006b) where AFLP-based diversities from 0.223 to 0.322 (mean HS of 0.27) were reported. Similar mean levels of diversity were found in *U. kirkiana* after applying RAPD DNA markers (Agufa 2002). In this study the mean diversity (HS) of 0.256, the AFLP-based estimates are quite similar to overall values obtained in studies where RAPDs were used (H range of 0.154 to 0.250, mean H = 0.192) (Agufa 2002), therefore suggesting that AFLP and RAPDs produce comparable results. The levels of diversity are also within the range reported for forest tree species of similar life history and reproductive ecology such as Sclerocarya birrea, Prunus africana and Vitex fischeri (Muok *et al.* 2007; Kadu *et al.* 2006; Lengkeek *et al.* 2006). The low genetic diversity in Mapanzure could be attributed to genetic drift because of small population size and forest fragmentation. Populations that are marginal or geographically isolated are expected to be more susceptible to the effects of genetic drift due to lower effective population size and/or increased selection pressure. Furthermore, positive phenotypic selection could result in possible shifts in allele frequencies leading to more loss of variation due to genetic drift. Like most tropical trees *U. kirkiana* is obligatory outcrossing and high intrapopulation variation revealed in the AMOVA confirms the general observation that differentiation is expected to be very restricted in long-lived woody, outcrossed and late successional species (Hamrick *et al.* 1992, Loveless and Hamrick 1984). Contrary to this, *U. kirkiana* is a pioneer tree species growing mostly in early successional habitats. Therefore the moderate population differentiation is caused mainly by obligatory outcrossing and longevity traits that are not typical for early successional species.

The use of morphological traits is not always the best way to evaluate genetic distance since the degree of divergence between genotypes at the phenotypic level is not necessarily correlated with a similar degree of genetic difference (Hamrick and Godt 1989). The lack of correlation between morphological and molecular markers shows that growth and morphological traits on their own are

less reliable and inefficient in analysis of genetic diversity.

The bulk of genetic diversity is contained within provenances but there is still appreciable differentiation among provenances. According to Wright (1978) F_{ST} values between 0.05 and 0.15 indicate moderate genetic differentiation (Hartl and Clark 1997). The mean F_{ST} value of the provenances was 0.089 suggesting that moderate differentiation can be attributed to both genetic drift, which increases differentiation, and gene flow, which reduces it. Dioecy in *U. kirkiana* implies obligate cross pollination, the plant is gregarious with sex ratio of mature trees in natural populations of about 1:1 (Ngulube *et al.* 1998). Since mature male and female individuals are spatially segregated and *U. kirkiana* is insect pollinated, although gregariousness reduces distances between dioecious plants long distance gene flow through pollen is expected to be low. A range of insects possible pollinators of *U. kirkiana* include bees (Apis mellifera) and beetles (Dothera bennigseni and Chelomenes lunata) which migrate only short distance (Ngulube *et al.* 1998). Monkeys and gorillas disperse fruits through dung to great distance places of over 200 km contributing high-quality dispersal to favourable sites over varied and changing environments (Voysey *et al.*, 1999). Furthermore, human transportation of fresh fruits across large distances between forests and towns improves germplasm exchange through seed dispersal within species natural distribution (Mwase *et al.* 2006b). The seed dispersal mechanisms would act as a homogenization factor between populations. Provenances from Zimbabwe showed higher levels of differentiation than provenances from Malawi, Zambia and Tanzania. This implies that the provenances of *U. kirkiana* cannot be considered a single panmictic unit although they are closely related. High within population variability is expected from such a plant with wide and continuous distribution (Hamrick and Loveless 1989). This may explain the limited genetic differentiation among provenances within countries although geographical distance between provenances varies from 95 - 480 km. Although there were no significant correlations between either morphological or AFLP-based differentiation with geographical distance between pairs of provenances, geographically close provenances were more similar than provenances from distant locations. Different levels of human impacts from origin site of collection might have large effects on genetic parameters measured and could explain the discrepancy between genetic and geographic distances. Certain AFLP alleles were present in certain populations only an indication of distinctiveness. This suggests that following long-term isolation by distance, partitioning of genetic variation in *U. kirkiana* is affected by effects of genetic drift, mutation rates and gene flow.

The provenance groupings accomplished through UPGMA were inconsistent with the geographical distribution of the studied provenances. Such results are consistent with the F-statistics for these areas, indicating that differences among countries have small implications in the distribution of genetic variation. Comparison of genetic diversity values between morphology and AFLP showed that the former ranged from 0.001 to 0.490, while the latter from 0.002 to 0.259 (data not shown). The correlations between the two genetic distances were low and non significant, indicating discrepancy between the two methods. Several comparisons between molecular and morphological studies also indicated that these two methods were different and highly variable (Roldan-Ruiz. *et al.*, 2001). This reflects the problem of morphological characterisation which is highly influenced by environment and different growth stages. The lack of correlations between the molecular and morphology could also be attributed to limited loci involved in morphology which may not be linked to any particular allele in AFLP, uneven marker coverage and non-coding sequences. However, such observations should not be regarded as indication of a weakness of these two methods, because genotypes that display high phenotypic similarity need not be genetically similar, as environment plays significant roles in phenotypic expression. This implies that AFLP could be important and reliable compared to morphological markers in assessing genetic diversity of plant species and this is corroborated by several authors (Barret and Kidwell 1998; Swanepoel 1999). While molecular markers show true expression of the genotype, morphological markers are still very useful in studying adaptive traits. While

acknowledging the importance of using local provenance for ecological adaptation our studies have shown that there is weak evidence that variation in average plant height among populations is associated with environmental gradients. High growth rates observed for provenances from Zambia and Zimbabwe suggests existence of high within provenance variability which allows the species to adapt to different environments without major genetic alteration.

The PCA results analysis shows that the twelve populations segregated into clusters consistent with results from morphological and AFLP derived dendrograms. The low percentage (22 %) of the variation explained by the first two components in the PCA could be attributed to high extent of genetic variability between individuals of each population relative to the low variation among the provenances. Although there were three clusters in the PCA only the cluster comprising of individuals from Mapanzure was distinct and this agrees with clusters obtained from UPGMA. The PCA also supports the dendrograms produced from morphological data, although there are some differences in the pattern of clustering. This has an important implication suggesting that it is difficult to predict the genetic diversity of populations based on morphological differences only. The PCA showed that Mapanzure was the most distinct and Nei's gene diversity showed least genetically diverse; however adaptive growth traits ranked Mapanzure as one of the best provenances and it was the only population that had started fruiting after 10 years of growth. It is recommended to study the fruit traits since its adaptive and genetic traits differ considerably from the rest of the provenances.

Strategies for prioritizing conservation of species diversity should consider the level of diversity as well as the existing threat of populations. The conservation in the different countries must consider the inevitable anthropogenic impacts of cultivation of forest lands on the distribution of genetic variation of the species. The populations with both low diversity and high diversity should be prioritized for conservation to serve as reservoirs of genetic variation and reduce genetic erosion after major changes in the habitat. Our results indicate that populations from Chipata, Choma and Murelwa harbour significant amount of diversity to warrant priority for conservation. The identification of moderate genetic variation have important implications for utilization of the species including the development of strategies for improvement of economically important traits such as fruit size, sweetness, colour, number of seeds and growth traits such as time to fruiting.

CONCLUSION

The analysis of genetic diversity of southern Africa provenances of *U. kirkiana* would assist in conserving the diversity of the species. The existence of higher level of variation within and moderate to low between populations suggests a vast genetic base available for selection and tree improvement. The high variation within *U. kirkiana* provenances suggests that sampling from a few populations within a country may capture a large proportion of variation. Nevertheless, sampling from a wide range of provenances among the countries is still advisable as there are significant morphological and AFLP differences between provenances and countries. Although morphological and growth characterisations are influenced by environment, they can be used hand in hand with molecular markers for revealing genetic variation. The significant variation in morphological and growth traits in *U. kirkiana* suggests the presence of adaptive genetic variation related to micro-environmental factors and there is great potential to establish high-diversity seed orchards. However when deciding on the most appropriate domestication and improvement strategy for *U. kirkiana*, heritability, vegetative propagation methods and genetic correlations between growth and desirable fruit traits such as time to first fruiting, fruit size, colour, sweetness and pulp ratio should be investigated.

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REFERENCES

Agufa, C.A.C., 2002. Genetic variation in Sclerocarya birrea and Uapaca kirkiana - indigenous fruit trees of the miombo woodlands. MSc thesis Jomo Kenyatta University of Agriculture and Technology, Nairobi Kenya.

Akinnifesi, F.K., Mhango, J., Sileshi, G. and Chilanga, T., 2008. Early growth and survival of three miombo woodland indigenous fruit tree species under fertilizer, manure and dry-season irrigation in southern Malawi. For Ecol Manage 255, 546-557.

Akinnifesi, F.K., Kwesiga, F., Mhango, J., Chilanga, T., Mkonda A., Kadu, C.A.C., Kadzere, I., Mithofer, D., Saka J.D.K., Sileshi, D., Ramadhani, T. and Dhliwayo, P., 2006. Towards the development of Miombo fruit trees as commercial tree crops in Southern Africa. Forests, Trees Livelihoods 16, 103 -121.

Akinnifesi, F.K., Kwesiga, F., Mhango, J., Mkonda, A., Chilanga, T. and Swai, R., 2004a. Domesticating priority miombo indigenous fruit trees as a promising livelihood option for smallholder farmers in southern Africa. Acta Hort 632, 15-30.

Akinnifesi, F.K., Chilanga, T.G., Mkonda, A., Kwesiga, F. and Maghembe, J.A., 2004b. Domestication of Uapaca kirkiana in southern Africa: Preliminary results of screening provenances in Malawi and Zambia. In Rao M.R., Kwesiga F.R. (eds). Agroforestry Impacts on Livelihoods in Southern Africa: Putting research into Practice. Aventurra Resorts Warmbaths, South Africa 20-24 May 2002, pp 85-91.

Barrett, B.A. and Kidwell, K.K., 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest Crop Sci 38, 1261-1271

Cao, C.P., Finkeldey, R., Siregar, I.Z., Siregar, U.J. and Gailing, G., 2006. Genetic diversity within and among populations of Shorea leprosula Miq. and Shorea parvifolia Dyer Dipterocaracaeae in Indonesia detected by AFLPs. Tree genetics and Genomes 2, 225 - 239.

Esbensen, K.H., 2007. Multivariate Data Analysis - in practice, 5th Ed, CAMO ASA, Oslo, 598pp.

Excoffier, L., Smouse, P.E. and Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 179-191.

Ham, C., Akinnifesi, F.K., Franzel, S., D. du P.S. Jordan, Hansmann, C., Ajayi, O. and C. de Cock, D., 2008. Opportunities for commercialization and Enterprise Development of Indigenous fruits of southern Africa. In: Akinnifesi, F.K., Leakey, R.B., Ajayi, O.C., Sileshi, G., Tchoundjeu, Z. Matakala, P. Kwesiga, F. 2008. Indigenous Fruit Trees in The Tropics. Domestication, Utilization and Commercialization. CAB International 438pp

Hamrick, J.L., Godt, M.J. and Sherman-Broyles, S.L., 1992. Factors influencing levels of genetic diversity in woody plant species. New For 6, 95-124.

Hamrick, J.L. and Loveless, M.D., 1989. The genetic structure of tropical tree populations: associations with reproductive biology. In: Bock J.H., Linhart, Y.B. (eds). The evolutionary ecology of plants. Boulder, CO, Westview Press, 129-146.

Hamrick, J.L., Godt, M.J.W., Murawski, D.A. and Loveless, M.D., 1991. Correlations between species trait and allozyme diversity:

Implications for conservation biology. In: Genetics and Conservation of Rare plants (eds) Falk DD, Holsinger KE) pp 75-86. Oxford University Press, Oxford.

Hartl, D.L., Clark, A.G., 1997. Principles of population genetics. 3rd edition. Sunderland (MA): Sinauer Associates.

Hodgetts, R.B., Aleksiuk, M.A., Brown, A., Clarke, C., Macdonald, E., Nadeem, S. and Khasa, D., 2001. Development of microsatellite markers for white spruce (Picea glauca) and related species. Theor Appl Genet 102, 1252-1258.

Kadu, C.A.C., Imbunga, M., Jamnadass, R. and Dawson, I.K., 2006. Genetic management of indigenous fruit trees in southern Africa: A case study of Sclerocarya birrea based on nuclear and chloroplast variation. South African J. Bot 72, 421-427.

Kremer, A., Caron, H., Cavers, S., Colpaert, N., Gheysen, L. and Gribel, R., 2005. Monitoring genetic diversity in tropical trees with multilocus dominant markers. Heredity 95, 274−280.

Leakey, R.R.B., Tchoundjeu, Z, Schreckenberg, K., Shackleton, S. and Shackleton, C., 2005. Agroforestry tree products. Targeting poverty reduction & enhanced livelihoods. Int J Agric Sust 3, 1-23.

Leakey, R.R.B., Tchoundjeu, Z, Smith, R.I., Munro, R.C., Fondoun, J.M., Kengue J., Anegbeh, P.O., Atangana, A.R., Waruhiu, A.N., Asaah, E., Usoro, E.C. and Ukafor, V., 2004. Evidence that subsistence farmers have domesticated indigenous fruits (Dacryodes edulis and Irvingia gabonensis) in Cameroon and Nigeria. Agrofor Syst 60, 101-111

Lengkeek, A.G., Mwangi, A.M. and Agufa, C.A.C., 2006. Comparing genetic diversity in agroforestry systems with natural forest: a case study of the important timber tree Vitex fischeri in central Kenya. Agrofor Syst 67, 293-300

Loveless, M.D. and Hamrick J.L., 1984. Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 15, 65-95

Maghembe, J.A., Simons, A.J., Kwesiga, F. and Rarieya, M., 1998. Selecting indigenous trees for domestication in southern Africa: priority setting with farmers in Malawi, Tanzania, Zambia and Zimbabwe. ICRAF. Nairobi, Kenya.

Maghembe, J.A., Kwesiga, F., Ngulube, M., Prins, H. and Malaya, F., 1994. Domestication potential of indigenous fruit trees of the Miombo Woodlands of Southern Africa. In: Leakey, R.R.B and Newton, A.C. (eds.) Tropical trees: Potential for Domestication and the Rebuilding of Forest Resources, pp. 220-229. HMSO, London, UK.

Mashingaidze, K., Musarirambi, M.T. ans Tongozha, P., 1991. Plant Genetic Resources Conservation in Zimbabwe. Mambo Press, Harare, pp. 9-15.

Mhango, J.L., 2000. Soil and nutrient requirements for early growth of Uapaca kirkiana seedlings. In: Akinnifesi F.K. *et al.*, (eds) Achievements in Agroforestry research and development in Malawi. Annual Report 2000. SADC-ICRAF, Harare, Zimbabwe, pp 62-67

Mwamba, C.K., 1995. Variations in fruits of Uapaca kirkiana and effects of in situ silvicultural treatments on fruit parameters: In Improvement of indigenous fruit trees of the miombo woodlands of southern Africa, pp. 50-57. ICRAF, Nairobi, Kenya.

Mwase, W.F., Bjørnstad, Å., Ntupanyama, Y.M., Kwapata, M.B. and Bokosi, J.M., 2006a. Phenotypic variation in fruit, seed and seedling traits of nine Uapaca kirkiana provenances found in Malawi. Southern African For 208, 15-21.

Mwase, W.F., Bjørnstad, Å., Stedje, B., Bokosi, J.M. and Kwapata, M.B., 2006b. Genetic diversity of Uapaca kirkiana Muell. Årg. populations as revealed by amplified fragment length polymorphisms (AFLPs). African J. Biotech: 5 (13), 1205-1213.

Muluvi, G.M., Sprent, J.I., Soranzo, N., Provan, J., Odee, D., Folkard, G., McNicol, J.W. and Powell, W., 1999. Amplified Fragment Length

Polymorphism (AFLP) analysis of genetic variation in Moringa oleifera Lam. Mol Ecol 8, 463-470.

Muok, B.O., Matsumura, A., Ishii, T. and Odee, D.W., 2007. Genetic diversity within Sclerocarya birrea populations in Kenya. J Arid Envir 71, 1-11.

Ngulube, M.R., Hall, J.B. and Maghembe, J.A., 1995. Ecology of a miombo fruit tree: Uapaca kirkiana (Euphorbiaceae). For Ecol Manage 77, 107-111.

Ngulube, M.R., Hall, J.B. and Maghembe, J.A., 1997. Fruit, seed and seedling variation in Uapaca kirkiana from natural populations in Malawi. For Ecol Manage 98, 209-219.

Ngulube, M.R., Hall, J.B. and Maghembe, J.A., 1998. Reproductive ecology of Uapaca kirkiana (Euphorbiaceae) in Malawi, southern Africa. J. Trop Ecol 14, 743-760.

Rohlf, F.J., 2000. NTSYS-pc. Numerical Taxonomy and Multivariate analysis system, version 2.1. Exeter Software, New York, USA.

Roldán-Ruiz, I, van Euwijk, F.A., Gilliland, T.J., Dubreuil, P., Dillmann, C., Lallemand, J, De Loose, M. and Baril C.P., 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (Lolium perenne L.) varieties Theor Appl Gen 103,1138-1150.

Schneider, S., Roessli, D. and Excoffier, L., 2000. Arlequin version 2.0: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sneath, P.H.A. and Sokal, R.R., 1973. Numerical Taxonomy. The Principles and practice of Numerical Classification, XV. Freeman, San Francisco, USA, pp 573.

Swanepoel, E., 1999. Identification of genetic distances and heterotic groups of inbred maize (Zea mays) lines using DNA fingerprinting. Department of Plant Breeding, University of the Orange Free State, Bloemfontein, South Africa.

Vos P., Hogers, R., Bleeker M., Reijans, M., van der Lee T, Hornes, M, Frijters, A., Pot, J., Peleman, J. Kuiper, M. and Zabeau, M., 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res 23, 4407-4414.

Voysey, B.C, Mcdonald, K.E., Rogers, M.E., Tutin, C.E. and Parnell, R.J., 1999. Gorillas and seed dispersal in the Lope´ Reserve, Gabon. II: Survival and growth of seedlings J. Trop Ecol 15, 38- 60.

Wright, S., 1978. Evolution and genetics of populations: Variability within and among natural populations vol 4, University of Chicago Press, Chicago, USA.

Appendix 1 : List of woody species found at Mgori forest reserve in Singida District, Tanzania

Botanical Name	Local Name	Botanical Name	Local Name	Botanical Name	Local Name
Acacia hockii	Munying'anyi	*Commiphora mosambiensis*	Muntonto	*Ozoroa insignis*	Munyongwampee
Acacia senegalsis	Mujighulu	Commiphora ngogensis	Mujuhu	*Pavetta schumanniana*	Munkuharii
Acacia sieberana	Mukese	*Commiphora ugogensis*	Musake	*Phylanthus ingleri*	Mubolomi
Acacia tortilis	Mughuunga	*Dalbergia melanoxylema*	Mufako	*Pleuurostylia africana*	Mufafati
Acaia tanganyikensis	Mughangachuma	*Dalbergia nitidula*	Mubibi	*Pramna senensis*	Munyukinyuki
Accacia drepandobium	Mwandui	*Dalbergia stulhmanii*	Musisi	*Pseudolachostylis maprouneifolia*	Muranghambili
Adansonia digitata	Mwandui	*Dichrostachys cinerea*	Mutunduru	*Pterocarpus angolensis*	Muhinga
Afzelia quanzensis	Mukola	*Diospyros usambarensis*	Muriyoriyo	*Pterocarpus rotundifolius*	Musalaka
Albizia antunesiana	Munyingafumbu	*Dolichos oliveri*	Mughongoafage	*Pyrenacantha kaurabassana*	Muiro
Albizia harvei	Mupogowa	*Erythrina abyssinica*	Mupipiti	*Schreberia tricoclada*	Muuma
Albizia petersiana	Musimihi	*Euphorbia candelabrum*	Mwange	*Sclerocarya birrea*	Muhuvi
Albizia zetersiana	Mpilo	*Ficus stuhlmanii*	Musaghaa	*Shrebera trichoclada*	Mwama
Azanza garckeana	Mutongho	*Greela arborea*	Mudoghwe	*Solanum incanum*	Mutula
Boscia angustifolia	Mutii	*Grewia platyclada*	Musuna	*Strychnos cocculoides*	Mukuhughundu
Boscia salicifolia	Muhuka	*Hymenodictyon parvifolium*	Mukumiankoo	*Strychrios potaforum*	Mupande
Brachystegia microphylla	Mukinki	*Isoberlinia angolensis*	Mukonjee	*Terminalia mollis*	Mughuka
Brachystegia spiciformis	Mufumbu	*Jubernadia globiflora*	Mufumbu 2	*Terminalia sericea*	Mufuru
Bridelia duvigneaudii	Musekea	*Kigelia africana*	Mugunghu	*Tricalysia ruandensis*	Muhuti
Canthium burtii	Musule	*Lannea humilis*	Muhinti	*Vangueria infausta*	Mulade
Cassipourea mollis	Mutuampiti	*Lannea schimperi*	Mughumbu	Vangueria madascaensis	Mukukutu
Catunaregam spinosa	Mupongwa	*Lonchocarpus bussei*	Muvae	*Vitex mombassae*	Musasati
Cissus rubiginosa	Mubwammwaka	*Margaritaria discoidea*	Museka	*Xeroderris stunhlmannii*	Mujimbua
Combretum collinum	Mufafage	*Markamia lutea*	Mughwanda	*Ximenia caffra*	Mutundwi
Combretum molle	Murama	*Markamia obtsifolia*	Mulili	*Zanha africana*	Mujijiu
Combretum obovatum	Mughianduata	*Multidentia crassa*	Mukukumaka		
Combretum zeyheri	Muhanyati	*Mundulea sericea*	Muheruheni		
Commellina beghalensis	Mungo'ngo	*Ormmocarpum trichocarpum*	Murori		
Commiphora africana	Mulalahai	*Ormocarpum trichocarpum*	Musimbwa		