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Identification of Specific Markers Linked to Regional Differentiation of *Warburgia ugandensis*

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KEYWORDS Random Amplified Polymorphic DNA. Amplified Fragment Length Polymorphism. Basic Local Alignment Search Tool. Multiple Sequence Alignment. Sequence

ABSTRACT *Warburgia ugandensis* is an important African medicinal tree. The species population has shown a high genetic differentiation in the Kenya's Rift Valley. Nine populations were analysed by Bulk Segregant Analysis employing Random Amplified Polymorphic DNA marker technique to identify regional differentiation-linked markers within and across Kenyan Rift Valley. Five primers showed putative East and West genetic differentiation. Diagnostic markers were isolated, cloned, sequenced and compared with Genbank sequences using BLAST algorithms. Three, (*WarburgiaIC15E, WarburgiaIC55E* and *WarburgiaIC28W*) sequences showed homology to plant and bacterial-like chromosomal sequences with low E-values. Sequence alignment indicated conserved protein domains of plants and bacteria-like sequences. Phylogenetic analysis revealed high rates of genetic distances (H" 0.8) and a low rate of disparity indices of (0), suggesting some evolutionary forces behind demographic differentiation. These imply that genetic differentiation observed might be due to genetic mutants in certain domains of chromosome that may have some implication on genome functionality.

INTRODUCTION

Warburgia ugandensis is an important medicinal tree belonging in the family Canellaceae that has restricted distribution in the tropical afro-alpine environment (Van Breugel et al. 2012). The tree faces a genetic disjunction created by evolutionary forces and possibly extinction consequences (Muchugi et al. 2008). The tree possesses medicinal compounds both antibacterial and antifungal properties and are widely used to cure several ailments such as malaria (Olila et al. 2001; Njoroge and Bussmann 2006; Nanyingi et al. 2008; Were et al. 2010). It's medicinal efficacy from the species extracts have been tested both *in vivo* and *in vitro* trials after isolating major bioactive ingredients (Rabe and Van-Staden 2000; Were et al. 2010). The phytochemical extracts discovered in *W. ugandensis* include alkaloid skimmianine and sesquiterpene muzigadial (Olila et al. 2001; Wube et al. 2005).

There is a great need to conserve the tree due to a genetic disjunction displayed by the populations across the Rift Valley so that unique regional germ-plasm is not lost through over exploitation (Muchugi et al. 2012). This strategy is important in selections planting material given that a superior germplasm within species are based on most conserved genes that show traits with great fitness (Upadhyaya et al. 2011). *Warburgia ugandensis* population from the West of the Kenyan Rift Valley and the rest of the population under study have showed high genetic differences which has been thought that perhaps are linked to its ecological range, that may implicate phytochemicals production (Muchugi et al. 2008).

The kind of genetic differences observed in the East of the Kenyan Rift Valley compared to its genetic diversity could have been brought about by the past historical and ecological consequences a cross the Rift Valley (Hughes et al. 2008; Scholz et al. 2011). Habitat fragmentation and decline in population size leading to genetic drift causing loss of important alleles in a stochastic fashion (Dixo et al. 2009). This kind

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of a genetic drift can weaken the survival ability of species populations consequently causing speciation (Guo et al. 2009). The kind of unique genetic disjunction seen in *W. ugandensis* had also been observed in two other indigenous tree species within the Rift Valley in Kenya *Prunus africana* (Muchugi et al. 2006) and *Lobelia giberroa* in Ethiopia (Muluget et al. 2007).

Distribution of important indigenous species in sub-Saharan Africa very much determined by evolutionary events, such as historical climatic contingencies and topographical features with mountain ranges acting as gene flow barriers (Muluget et al. 2007). Plant populations at the afro-alpine belt experience low altitudinal range shifts compared to latitudinal, with elevated climatic gradients bearing species losing their genetic diversity therefore, attributing to repeated species relation bottlenecks (Weber and Schmid 1998; Muluget et al. 2007). Despite all these, mountains of the afro-alpine Tropical to East Africa have provided a relatively stable habitat with older species surviving by altitudinal range shifts while new lineages are generated (Finch et al. 2009). The long-term environmental stability of the region's forest has been proposed to be of an accumulation and persistence mechanism of species during glacial periods that have resulted in diverse species assemblages (Finch et al. 2009).

This has therefore made the mountains in this region an ideal model system for studying speciation and adaptation of plants due to ecological and geographical modifications (Bussmann 2006). Even though moisture availability and the usual altitudinal lapse rate control most of the vegetation patterns, the plant communities in the region depend on edaphic modification components (Linder et al. 2005). Population densities of *W. ugandensis* tree population in this region have been found to be small in the ecological survey, though their genetic differentiation was high within the populations as compared to other molecular studies on other afro-alpine indigenous tree species (Dawson and Powell 1999; Muchugi et al. 2008). Given that habitat fragmentation, and decline in population size can lead to a significant genetic drift Guo et al. (2009), an amount of genetic differentiation among populations can also provide useful data for planning actions such as reinforcement of existing populations, reintroduction of *ex situ* culture or seed collection to conserve genetic diversity (Petit et al. 1998).

Genetic differentiation through Marker Assisted Selection (MSA) such Random Amplified Polymorphic DNA RAPD technique is the method of choice in profiling data to be considered when planning plant conservancy plans. This method has fascinated evolutionary scientists for its economic prominence through conservation of plant species for sustainable utilisation in healthcare and agriculture (Malla 2008; Jamnadass et al. 2009; Cochrane 2011). RAPD markers are amplicons of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence (Tulsiani et al. 2010). Carefully developed laboratory protocols have been developed to overcome any inherent problems such as low reproducibility of results and changes in reaction conditions of RAPD-PCR analysis (Das et al. 2009). The technique is therefore, an ideal marker system in conservation for it can establish the genetic basis of important traits in plant accessions to facilitate efficient management of germplasm collections (Kumar et al. 2011; Upadhyaya et al. 2011). Visualization of generated bands on excised gels is easy, these bands can therefore, be purified, cloned, transformed, sequenced and finally analysed using bioinformatics tools.

The study aimed at identifying specific adaptative regional genetic markers in *W. ugandensis* of the Kenyan Rift Valley. This is because marker assisted selection in addition to other techniques such as Bulk Segregant Analysis has been used to provide novelty in the genome studies (Michelmore et al. 1991; Sestraset et al. 2009). In a previous study on *W. ugandensis* genetic structure and *L. giberroa* genetic differentiation studies showed evidence of diagnostic markers (Muluget et al. 2007; Muchugi et al. 2012). Using the logic that related organisms generally have a high degree of agreement in the molecular structure as opposed to distantly related organisms that show a pattern of dissimilarity, this theory was used to distinguish the genetic differences in *W. ugandensis* (Liao et al. 2010). Therefore, RAPD technique was used to reveal the existence of the diagnostic markers that show differentiation which then were sequenced for genetic analysis.

Phylogenetic trees were reconstructed to provide information on the diagnostic sequences of the species evolution through genetic distance

and disparity indices (Kumar and Gadagkar 2001; Tamura et al. 2007). The phylogeny were also reconstructed to predict protein-protein interactions, protein structure determination, estimation of divergence time and predicting gene functions through BLAST (Chen et al. 2004). Genetic distance and disparity indices studies were used to compare the genetic similarity and measures of divergence between different plant species and sub-species (Khan et al. 2011).

MATERIAL AND METHODS

Sampling of Populations

Leaf samples collected from 20 trees at a minimum distance of about 100 m apart from eight populations of *W. ugandensis* from the Kenyan side of the Eastern Great Rift Valley were used. Sampled locations were selected to cover a wide range of the natural distribution of the species, with a focus on political administrative county units within the Kenyan Rift Valley region shown in Table 1. The samples from Uganda and Tanzania were included as reference samples to assess the wider-scale differentiation in the bulked samples from the previous studies shown in Figure 1 (Muchugi et al. 2008). One sample from Londiani was also introduced as a test sample because the location is at the midpoint of the East and West regions of the Great Rift Valley.

Genomic DNA extraction was done using Cetyl trimethylammonium bromide (CTAB) based protocol (Doyle and Doyle 1987). The DNA concentration of each sample was estimated by visual assessment relative to phage DNA molecular scale of different known concentrations on 1% (w/v) agarose gel (Sambrook et al. 1989). Genomic DNA was diluted in a tube using sterile triple distilled water to a final concentration of approximately 2.5 ng/µl for bulking. Two bulks through BSA were established representing East and West population regions of the Rift Valley. For each bulk, 12.5 ng of DNA was used. The bulked DNA was gently vortexed and centrifuged to establish a homogenous DNA mix. These dilutions were stored at -20°C until required.

For PCR amplification each 20 iL PCR contained 1× PCR buffer, 2 mM $MgCl_2$, 200 iM of each dNTP, 0.13 iM of each primer. Table 2 shows the primers used in the study, 0.5 U *Taq* DNA polymerase (Amplitaq Gold™; Applied Biosystems), and ~40 ng template DNA. Thermocycling conditions PTC-200 (MJ Research Inc.) were: Initial denaturation step of 15 minutes at 94°C, followed by 45 cycles of one minute at 94°C, one minute at 30°C, two minutes at 72°C with a further final extension step of 10 minutes at 72°C. The reproducibility of RAPDs was tested by repeating a subset of samples across PCR runs.

Table 1: RAPD-PCR Loading order from of samples

Lane	*East and West regions of the Rift Valley*	*Sampled populations*
M	-	100 bp ladder (Promega, Madison-USA)
1	West	Bulked samples; Kitale1 + Cherangani
2	West	Bulked samples; Kitale2 + Cherangani
3	West	Bulked samples; Kitale4+ Kibale
4	West	Individual sample as control; Cherangani
5	East and within	Individual sample as control; Londiani
6	East and within	Bulked samples; Karura1 + Laikipia
7	East and within	Bulked samples; Karura2 + Laikipia
8	East	Bulked samples; Karura + Taita
9	East and out of the R.V	Bulked samples; Laikipia + Lushoto

Order of loading DNA and the molecular ruler in the agarose gel wells by separating the representative regions as bulked East and West sample populations.

Table 2: Oligonucleotide primers that showed differentiation

Primer	*Sequence*
IC11	5'GCATGGAGCT-3'
IC15	5'GGGACGTCTC-3
IC21	5'GCTAGGGCGG-3'
IC28	5'CCCGGCTTGT-3'
IC55	5'GTAGACCCGT-3'

The oligonucleotides (Operon primers) used for the population differentiations in *W. ugandensis* and the sequences.

A total of 20 µl PCR products plus 1 ìl of Bromophenol blue in xylene cyanol loading dye were loaded in a 2% (w/v) agarose gel and resolved by electrophoresis in 1 X TBE buffer at 5 V/cm for three and half hours on 2% (w/v)

Fig. 1. Geographical locations of *W. ugandensis* material used. The map is a representation of the sampled regions along and within the Rift Valley

agarose gel in a submarine Electrophoretic tank. The band products were sized against 100 bp ladder (Promega, Madison-USA). Visualisation was done under UV light after staining for 30 min in 0.5 ig/ml of ethidium bromide in 300 ml of TBE buffer, and the gel was photographed using UVI gel documentation system (UVI Gel documentation, USA). Fragments exclusively present on the samples from the East and West on agarose gel electrophoresis that showed relatively simple, clear, and reproducible banding patterns were selected and cut out with a sterile scalpel for further studies. The PCR products were excised, purified and eluted from the gel slices using a QIAquick® gel extraction kit (QIAgen Inc., Valencia, CA) according to the manufacturer's instructions. The purified eluted fragments were then suspended in 10 µl of 10 mM Tris HCl pH 8 and stored at 4°C awaiting cloning. To show sensitivity of the RAPD markers 2 replicate gels after several primer screening were also used in the study to show consistency of their amplified characterised regions for cloning and sequencing.

Cloning, Plasmid DNA Preparation and Sequencing

Cloning of the recovered purified DNA was done in a total reaction volume of 10 µl, ligated into 1 µl pGEM-T easy vector (Promega) in *E. coli* (DH5á) cells. Plasmids DNA were purified using the QIAprep Spin Miniprep Kit (QIAGEN) and sequenced by BigDye Terminator Cycle chemistry using M13F-pUC primers and an ABI 3800xl DNA Analyser (Applied

Biosystems). Recombinants were analyzed by PCR using universal primers (M13) that flank the multiple cloning sites and allow direct sizing of the PCR products primers (Messing 1983). The PCR cocktail was prepared consisting 5 µl of 10 X PCR buffer, 0.5 µl of 50 mM dNTPs, 1 µM of 100ng/µl M13 primers and 1 U of DNA *Taq* Polymerase. The total PCR mix was made to 20 µl with DNA added as template by touching the clones (white colonies) to confirm true transformants. The Plasmid DNA were then purified using QIAquick® gel extraction kit [QIAgen Inc., Valencia, CA] according to manufacturer's protocol.

Sequence Analysis

The purified plasmids DNA were subjected to DNA sequencing with ABI 3100 Genetic analyser using (Applied Biosystems). Squences were edited by Vecscreen from (NCBI) and sequence scanner software (http://bioinformatics.unc.edu/software/sequencher/seq_ABI_files_mac.htm) *software for* edits.

Sequence Comparison from the Genbank

The sequences were compared against the Gen-bank non-redundant protein database National Center for Biotechnology Information (NCBI) using the BLASTX and TBLASTX algorithms (Altschul et al. 1990). The generated amino acid sequences were queried with the higher plants and other organisms' database at the NCBI to determine E-values at a statistical threshold of 0.0001. Nucleotide sequences were translated to protein using at Swiss Bioinformatics Institute website – Expasy (http://us.expasy.org) (Bairoch 1991). The significant amino acids resultant sequences were compared through pair-wise alignments with ClustalW (Altschul et al. 1997; Thompson et al. 2011) at http:www.ebi.ac.uk/clustalw/, and edited by biological sequence alignment editor- BioEdit (Hall 1999) and Needleman-Wunsch global alignment (NeedleN) (Needleman and Wusch 1970; Rice et al. 2000). The amino acids were used to query various public protein databases to identify conserved domains that included protein domains, families and functional sites in InterPro (Apweiler et al. 2000; Hulo et al. 2004; Mulder et al. 2005) and their bioinformatics data bases found at http://www.ebi.ack.uk/interpro, prosite of http//au.expasy.org/tools/scanprosite, Pfam of http://pfam.wustl.edu/hmmsearch.shtml (Bateman et al. 2004; Sonnhammer et al. 1998) databases.

Phylogenetic Analysis

Genetic relationships and differentiation were determined by phylogenetic analysis through a uniform weighted parsimony method using Molecular Evolutionary Genetics Analysis (MEGA3) software of (http://www.megasoftware.net/mega.html).

RESULTS

Identification of RAPD Markers Showing East and West Differentiations

DNA with good quality and quantity from the yield extracted was used for PCR amplifications to obtain RAPD polymorphic bands. A total of 20 RAPD primers sets were tested. Only five primers sets showed clear banding pattern with good resolution of East and West population differentiation in the Kenyan Rift Valley shown in Figures 2 and 3 with populations 4 showing very faint bands for both East and west populations. The results in the study showed western populations differences compared using three RAPD primers IC21 IC28 and IC55 revealing the banding pattern differences. Primer IC21 and IC55 showed there were band differences in the populations seen in the West though there appeared some faint bands in the populations seen on bands 4 probably suggest DNA degradation, IC21 produced the bands at 300 bps shown in Figure 2. Eastern populations' differentiations were only observed with a RAPD primer IC28 showing two different band levels at (380 bps and 750 bps) as shown in Figure 3.

W. ugandensis homologous sequences found in the NCBI similarity searches by TBLASTX revealing species, sequences genbank accession numbers, the locus at which they were found, the expected length of the sequences, E-values and identities as shown in Table 3. A multiple sequence alignment of these sequences and *W. ugandensis* consensus sequences showed some significant alignments in as seen in Figure 4 and the results showed higher alignment scores in as seen in Table 4. The alignments features were explained in the MSA chart as, amino ac-

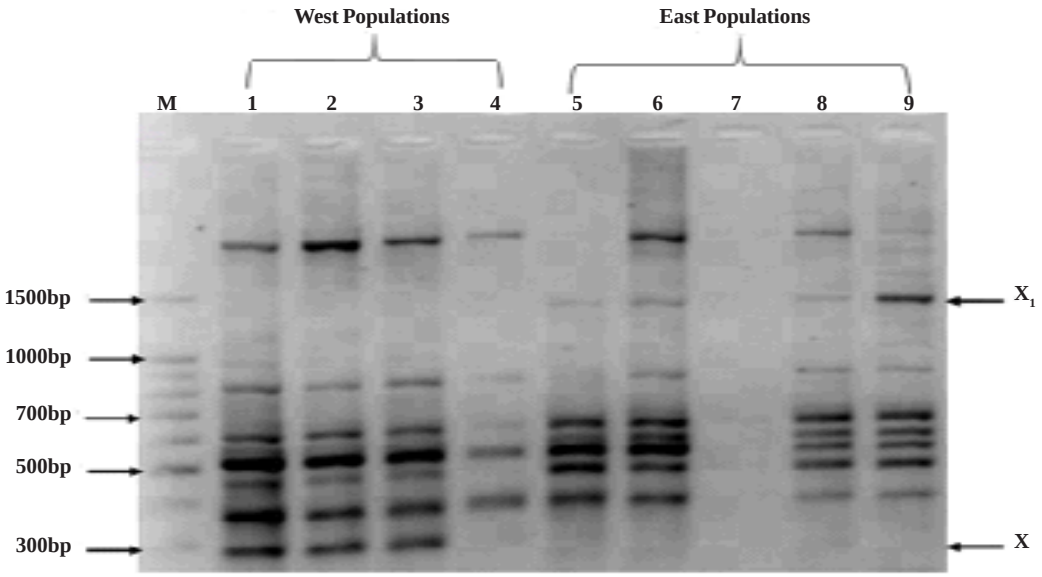

Fig. 2. RAPD profiles of *W. ugandensis* using primer IC21.
X and X_1 arrows show RAPD bands revealing population differences, while M is a molecular size maker of 100 bps (Population 1 Kitale1 + Cherangani, 2 Kitale2 + Cherangani, 3 Kitale4+ Kibale, 4 Cherangani, 5 Londiani, 6 Karura1 + Laikipia, 7 Karura2 + Laikipia, 8 Karura1 + Taita, 9 Laikipia + Lushoto) **.**

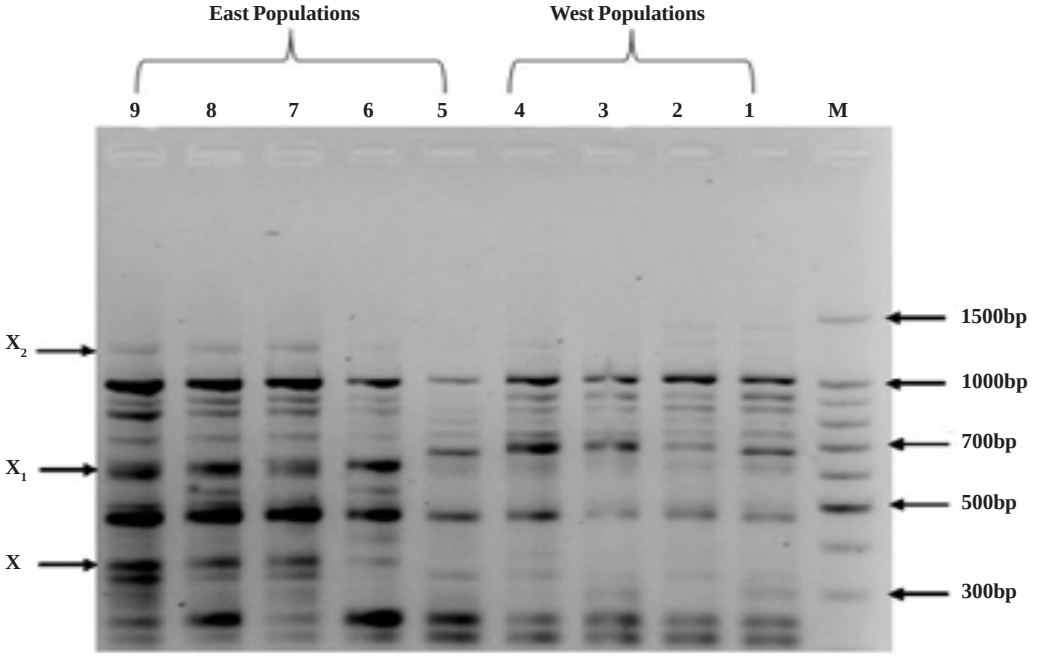

Fig. 3. RAPD profiles of *W. ugandensis* using primer IC28.
X and X_1 arrows show RAPD bands revealing population differences, while M is a molecular size maker of 100 bps (Population 1 Kitale1 + Cherangani, 2 Kitale2 + Cherangani, 3 Kitale4+ Kibale, 4 Cherangani, 5 Londiani, 6 Karura1 + Laikipia, 7 Karura2 + Laikipia, 8 Karura1 + Taita, 9 Laikipia + Lushoto). X_2 is a region of that show very faint differential markers that were not considered

Table 3: TBLASTX *Warburgia ugandensis* sequence NCBI similarity searches

Homologues	*GenBank Accession No.*	*Locus*	*Expected size*	*E-value*	*Identity*
Artemisia annua strain Artemis	EZ153264.1	EZ153264	402 aa	1e-19	100%
Collimonas fungivorans	AY593480.1	AY593480	11502 aa	5e-16	90%
Candidatus Glomeribacter gigasporarum	AJ561042.3	AJ561042	946 aa	2e-14	86%

Table 4: Multiple sequence alignment of *Warburgia ugandensis* and 3 significant homologous sequences

SeqA	*Name*	*Len(aa)*	*SeqB*	*Name*	*Len(aa)*	*Score*
1	*Warburgia*	264	2	*Artemisin*	402	43
1	*Warburgia*	264	3	*collimonas*	811	35
1	*Warburgia*	264	4	*Candidatus*	945	47
2	*Artemisin*	402	3	*collimonas*	811	51
2	*Artemisin*	402	4	*Candidatus*	945	56
3	*collimonas*	811	4	*Candidatus*	945	61

ids and their class. A reconstructed phylogenetic tree showed genetic distances and disparity indices as shown on Figure 5 and explained in Table 5.

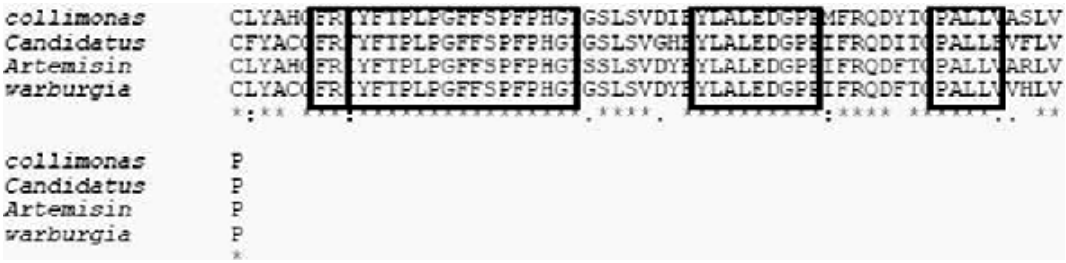

Fig.4. Alignments of the 3 *W. ugandensis* and significant retrieved homologous sequences in the study sequences submitted to NCBI accession numbers AY593480, AJ561042.3, EZ153264.1 in order as from above to *W. ugandesis* sequence homologs. (The symbols represents: (-) means many gaps, (":") very few conserved substitutions, (".") semi-conserved substitutions and ("*") very few identical amino acids.)

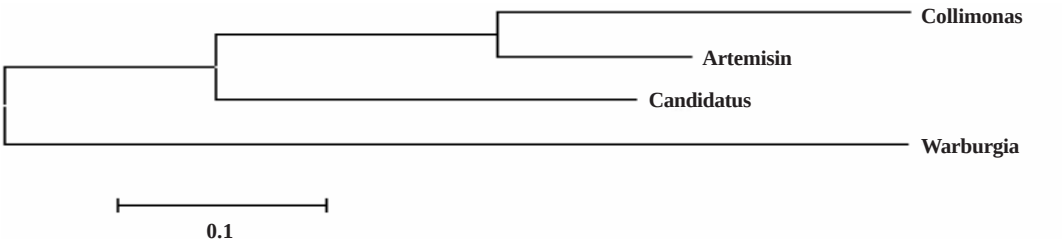

Fig. 5. Phylogram of the homologous sequences to all *W. ugandensis* significant sequences genetic pair-wise distance values of between 0.7 – 0.9

Table 5: Pairwise distances for all sequences and disparity pattern index in brackets

		1	*2*	*3*		*1*	*2*	*3*	*4*
1	*Collimonas*				*Collimonas*		(0.2)	(0.1)	(0)
2	*Artemisin*	0.5			*Artemisin*			(0)	(0)
3	*Candidatus*	0.3	0.4		*Candidatus*				(0)
4	*Warburgia*	0.9	0.7	0.8	*Warburgia*				

The phylogenetic relationships of the homologous sequences to those of *W. ugandensis* in terms of distance and the sequenced disparity indices.

The analysis results revealed two clusters with *W. ugandensis* sequences. The results were of significant higher genetic pair-wise distance values of between 0.7 - 0.9 when a consensus of all the three most significant sequences found from the *W. ugandensis* DNA analysed as (*WarburgiaIC55E, WarburgiaIC15W* and *WarburgiaIC28W*) that were earlier mined by NCBI and translated then aligned to its homologous sequences. While the homolog's disparity indices were of quit low levels of substitutions (0) that predicts bigger conservations within the protein domains along the alignments.

DISCUSSION

The differentiated RAPD bands reaveled clear diagnostic markers for the East and West regional differentiation within the species in the Kenyan Rift Valley. The introduced replicates RAPD-PCR of the same primer products did not show significant reproducibility of bands except for the discriminate bands. Patterns of differences were based on either the presence or absence of an allele in one region as opposed to the other. There were a few cases where RAPD amplified bands appeared faint but could be distinguished as discriminates this probably suggested DNA degradation in a population. This could have been brought about by how the DNA was stored from the previous studies.

From the five sets of oligonucleotide primers only three used in the study produced significant biological similarities with the lowest E-values for east and west differentiations. The homologous sequences found in the NCBI genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) through BLAST searches supported both the statistic E-values between 0.000-4e-33 and high percent sequence identity. Only 3 sequences known as *WarburgiaIC55W*, *WarburgiaIC15W, WarburgiaIC28E* and their consensus sequences (*WarburgiaIC55E, WarburgiaIC15W* and *WarburgiaIC28W*) from MSA retrieved revealed East and Western significant homolog's to plant and bacterial like sequences through BLASTX and TBLASTX.

Sequence *WarburgiaIC55W* revealed plants like homologs to *Artemisia annua* strain *Artemis*, *Siparuna decipiens* and *Climacium americanum.* The only sequence *WarburgiaIC28E* revealed bacterial like sequences homologs to *Arthrobacter sp. FB24*, *Rhodococcus erythropolis* and *Streptomyces species*. The consensus sequences of the significant *W. ugandensis* (*WarburgiaIC55W, WarburgiaIC11W* and *WarburgiaIC28E*) revealed both plant and bacterial like sequences of *Artemisia annua* strain *Artemis*, *Collimonas fungivorans fosmid* and *Candidatus Glomeribacter gigasporarum.* The amino acid sequences obtained from Expasy (http://us.expasy.org) and NCBI searches revealed some homology with protein functions in some regions of chromosome inform of transposable elements therefore, revealing a kind of divergence in the species.

The MSA of CLUSTALW (http://www.ebi.ac.uk/clustalw/) amino acid substitution matrices revealed significant alignments of *WarburgiaIC55W*, *WarburgiaIC11W* and *WarburgiaIC28E*. The only significant pair-wise sequences alignment were between *W. ugandensis* consensus sequences (*WarburgiaIC55E, WarburgiaIC15W* and *WarburgiaIC28W*) showing long conserved amino acid domains. Most of the amino acid sequences were either substituted by one another or gaps were added for a penalty. Most of the amino acid sequences were conserved to one another, with very few semi conserved regions. The highest scored alignments were between *W. ugandensis* with *Candidatus* and *Artemisia* sequences. *W. ugandensis* also had two orthologs namely *Climacium americanum* and *Populus balsamifera* subsp. *trichocarpa*. The species had ten paralogs namely *Siparuna decipiens*, *Gossypium*, *Physcomitrella patens subsp. Patens*, *Ricinus communis*, *Arthrobacter sp. FB24*, *Rhodococcus erythropolis*, *Streptomyces* strain, *Collimonas fungivorans fosmid*, *Artemisin annua* strain *Artemis* and *Candidatus Glomeribacter gigasporarum*.

Significant results from the phylogenetic analysis relationship revealed 3 clusters; the one between *W. ugandensis* and *Collimonas and Artemisin* showing a low genetic distance and a lower disparity index (rates of amino acid substitutions) of 0.5 and (0.2) respectively. The other cluster was between *Candidatus* and the first cluster of *Collimonas and Artemisin* showing a genetic distance and a disparity index between *Candidatus* and *Artemisin* as 0.4 and (0) respectively. These were also high enough to suggest a gene function between the two sequences. Another cluster analysis was between *Candidatus* and *Collimonas* showing a genetic distance of 0.3 and a disparity index of (0.1).

W. ugandensis sequence compare to the 3 sequences showed high genetic distance (as high as) 0.9 but very low disparity index of (0).

The kind of homologous sequence similarity functions retrieved were not sufficient enough to suggest *W. ugandensis* specific marker traits of adaptation within the Kenyan Rift Valley. Given that the rate of evolution of the sequences compared in the phylogenies is high with genetic distances (of an average 0.8) and low disparity indices of (0), suggests some evolutionary forces behind the demographic differentiation (Qui et al. 2006; Rensing et al. 2008). These kind of high genetic distances but low disparity can be as a result of mutational-variation within a particular gene or alleles in a given population (Young et al. 1996). Simple geographical isolation is not seen as the only widespread cause of speciation or interruption of gene flow between populations (Mallet et al. 2009). These sequences could be those of Transposable elements since the elements can be subjected to weak selection since their efficacy varies as a function of demographic factors, which is a major driving force on their distribution in the genome (Lockton et al. 2008). Other recent phylogeographic studies on *Banksia hookeriana, Farfugium* (Asteraceae) and *Prunus Africana* He et al. (2010) showed that the genetic structure of *Banksia hookeriana* suggest that tographical features may not be the reasons of species differentiation (He et al. 2010: Nomura et al. 2010: Kadu et al. 2011). The events might have been introduced by uninformative DNA sequence variation coupled with highly divergent morphologies for adaptive diversification as rapid (Hurst and Warren 2001; Heslop-Harrison 2010). The results did not find any link of the sequences to *W. ugandensis* sequences, though not enough in term of its full genome sequence has been done and with only closely related species showing low genome sequences (Muge et al. 2009; Kadu et al. 2011).

CONCLUSION

In conclusion, the study did not reveal RAPD-PCR genetic markers that distinguish *W. ugandensis* species populations either East or West categorised regions or a disjunction in the Kenyan Rift Valley. Although there were also some repeatable sequence variations between East and West populations, these were not specific genome markers. It is possible that the extent of genetic variation is only incipient, reducing the random chance that RAPD-PCR primers would amplify the variable regions, or that variation does not follow the geographical sampling pattern we adopted. Fine-scale markers would clarify this issue. The study suggests that they might have been some chromosomal insertions probably in the form elements such as transposable element's. The results observed from the significant homologous sequences similarity analysis suggest presence of transposable elements within the genome. The findings provide a basis for targeted study of fine-scale diversity such as through chloroplast DNA, transcriptome sequencing and analysis, Sequence Characterised Amplified Regions (SCARS) and Genome walking.

RECOMMENDATIONS

Though, the study did not reveal any association with a continual genetic disparity describing the Rift Valley as a gene flow barrier among *W. ugandensis* species. There is need to assess the active ingredients in *W. ugandensis* extracts associated with the herbal therapy across the species range in Kenya to establish whether there is any positive correlation to the genetic dynamics or edaphic factors.

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REFERENCES

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ 1997. Gapped BLAST and PSI-

BLAST: A new generation of protein database search programs. *Nucleic Acids Research,* 25: 3389-3402.

Altschul SF, Warren G, Webb M, Eugene W, Myers DJL 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215: 403 - 410.

Apweiler R, Attwood TK, Bairoch A, Bateman A, Birney E, Biswas M, Bucher P, Cerutti L, Corpet F, Croning MD, Durbin R, Falquet L, Fleischmann W, Gouzy J, Hermjakob H, Hulo N, Jonassen I, Kahn D, Kanapin A, Karavidopoulou Y, Lopez R, Marx B, Mulder NJ, Oinn TM, Pagni M, Servant F, Sigrist CJ, Zdobnov EM 2000. The InterPro-database, an integrated documentation resource for protein families, domains and functional sites. *Nucleic Acids Research*, 29: 3- 40.

Bairoch A 1991. SEQANALREF: A sequence analysis bibliographic reference databank. *Computational Application in Biosciences*, 2: 286.

Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Grffith-J, Khanna A, Marshall M, Moxon S, Sonnhammer E, Studholme D, Yeast C, Eddy S 2004. The Pfam protein families database. *Nucleic Acids Research,* 32: 138-141.

Bussmann RW 2006. Vegetation zonation and nomenclature of African mountains - An overview. *Lyonia Journal of Ecological Application,* 11: 41-66.

Chen W, Ortý G, Meyer A 2004. Novel evolutionary relationship among four fish model systems. *Trends in Genetics*, 2: 424-431.

Cochrane M 2011. The Fate of Alpine Species in the Face of Climate Change: A Biogeographic Perspective, Macalester Reviews in Biogeography, 2, Article 1. From <http://digitalcommons.macalester.edu/biogeography/vol2/iss1/1/>

Das BK, Jena RC, Samal KC 2009. Optimization of DNA isolation and PCR protocol for RAPD analysis of banana / plantain (Musa spp.) *International Journal of Agriculture Sciences,* 1(2): 21-25.

Dawson IK, Powell W 1999. Genetic variation in the Afromontane tree *Prunus africana*, an endangered medicinal species. *Molecular Ecology,* 8: 151-156.

Dixo M, Metzger JP, Morgante JS, Zamudio KR 2009. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation,* 142: 1560–1569.

Doyle JJ, Doyle JL 1987. CTAB DNA extraction in plants. *Phytochemical Bulletin*, 19: 11-15. Enfield, USA.

Dvorak JT 2009. Genome structure and evolution. In: C Feuillet, GJ Muehlbauer (Eds.): *Genetics and Genomics of the Triticeae*. USA: Springer, pp. 685-711.

Finch J, Leng MJ, Marchant R. 2009. Late Quaternary vegetation dynamics in a biodiversity hotspot, the Uluguru Mountains of Tanzania. *Quaternary Research*, 72: 111-122.

Guo Y, Bechsgaard J, Tanja S, Barbara N, Martin L, Detlef W, Schierup MH 2009. Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences of the United States of America*, 106: 5246-5251.

Hall TA 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series,* 41: 95-98.

He T, Lamont BB, Krauss SL, Enright NJ 2010. Genetic connectivity and inter-population seed dispersal of *Banksia hookeriana* at the landscape scale. *Annals of Botany*, 106: 457- 466.

Heslop-Harrison JSP 2010. Genes in evolution: The control of diversity and speciation. *Annals of Botany*, 106: 437-438.

Hughes RA, Inouye BD, Johnson MTJ, Underwood N, Vellend, Mark K 2008. Ecological consequences of genetic diversity. *Ecology Letters*, 11: 609 - 623.

Hulo N, Sigrist A, Le Saux V, Langendijk-Genevaux S, Bordoli L, Gattiker A, De Castro E, Bucher P, Bairoch A 2004. Recent improvements to the PROSITE database. *Nucleic Acids Research*, 32: 134 -137.

Hurst GDD, Werren JH 2001. The role of Selfish genetic elements in eukaryotic evolution. *Nature Reviews,* 2: 597- 606.

Jamnadass R, Lowe A, Dawson IK 2009. Molecular markers and the management of tropical trees: The case of indigenous fruits. *Biomedical and Life Sciences Tropical Plant Biology*, 2: 1-12.

Kadu CAC, Schueler S, Konrad H, Muluvi GMM, Eyog-Matig O, Muchugi A, Williams VL, Ramamonjisoa L, Kapinga C, Foahom B, Katsvanga C, Hafashimana D, Obama C, Geburek T 2011. Phylogeography of the Afromontane Prunus Africana reveals a former migration corridor between East and West African highlands. *Molecular Ecology*, 20: 165 -178.

Khan AI, Khan AI, Awan FS, Sadaqat HA, Bahadur S 2011. Estimation of genetic distance based on RAPDs between 11 cotton accessions varying in heat tolerance. *Genetics and Molecular Research*, 10: 96 -101.

Kumar J, Choudhary AK, Solanki RK, Pratap A 2011. Towards marker-assisted selection in pulses: A review. *Plant Breeding,* 130: 297-313.

Kumar S, Gadagkar RS 2001. Disparity index: A simple statistic to measure and test the homogeneity of substitution patterns between molecular sequences. *Genetics*, 158: 1321-1327.

Liao B, Liao B, Sun X, Zeng Q 2010. A novel method for similarity analysis and protein sub-cellular localization prediction. *Bioinformatics*, 1: 2678-2683.

Linder HP, Lovett J, Mutke JM, Barthlott W, Jürgens N, Rebelo T, Küper W 2005. A numerical re-evaluation of the sub-Saharan *phytochoria* of mainland Africa. *Biologiske Skrifter,* 55: 229 -252.

Lockton S, Ross-Ibarra J, Gaut BS 2008. Demography and weak selection drive patterns of transposable element diversity in natural populations of *Arabidopsis lyrata*. *Proceedings of the National Academy of Sciences of the United States of America*, 105: 13965 -13970.

Malla G 2008. Climate change and its impact on Nepalese agriculture. *The Journal of Agriculture and Environment*, 9: 62 - 68.

Mallet J, Meyer A, Nosil P, Feder JL 2009. Space, sympatry, and speciation. *Journal Evolution Biology,* 22: 2332-2341.

Maris C, Dominguez C, Allain F H 2005. The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression. *Febs Journal*, 272: 2118-2131.

Michelmore R, Paran I, Kesseli R 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences of the United States of America,* 88: 9828-9832.

Miller WJ, Capy P 2004. Mobile genetic elements as natural tools for genome evolution. *Methods in Molecular Biology*, 260:1-20.

Muchugi A, Kindt R, Muluvi G, Muge E, Kipruto H, Jamnadass HR 2012. Genetic variation of Kenyan populations of *Warburgia ugandensis*, an important East African Highlands medicinal tree species. *Journal of Life Sciences,* 4(2): 97-105.

Muchugi A, Muluvi GM, Kindt R, Kadu CAC, Simons AJ, Jamnadass RH 2008. Genetic structuring of important medicinal species of genus *Warburgia* as revealed by AFLP analysis. *Trees Genetics and Genome,* 4: 787-795.

Muchugi AN 2007. Population genetics and taxonomy of important medicinal tree species of the genus *Warburgia*. PhD Thesis in Molecular Genetics. Kenyatta University, Nairobi, Kenya, P. 134.

Muge E, Burg K, Kadu C, Muchugi A, Lemurt S, Jamnadass R 2009. Isolation of high quality DNA and RNA from cambium of the East African Greenheart (Warburgia ugandensis). *African Journal of Biotechnology,* 8 (13): 3036-3040.

Mulder NJ, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, Bradley P, Bork P, Bucher P, Cerutti L, Copley R, Courcelle E, Das U, Durbin R, Fleischmann W, Gough J, Haft D, Harte N, Hulo N, Kahn D, Kanapin J, Mitchell A, Nikolskaya AN, Orchard S, Pagni M, Ponting CP, Quevillon E, Selengut J, Sigrist CJ, Silventoinen V, Studholme DJ, Vaughan R, Wu CH 2005. InterPro, progress and status in 2005. *Nucleic Acids Research*, 1: 201-205.

Mulugeta AK, Dorothee E, Pierre T, Sileshi N, Christian B 2007. Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and Tropical East African mountains. *Molecular Ecology,* 16: 1233-1243.

Muotri AR, Marchetto MCN, Coufal NG, Gage FH 2007. The necessary junk: New functions for transposable elements. *Human Molecular Genetics,* 16: 159-167.

Nanyingi M, Mbaria J, Lanyasunya A, Wagate C, Koros K, Kaburia H, Munenge R, Ogara W 2008. Ethnopharmacological survey of Samburu district, Kenya. *Journal of Ethnobiology and Ethnomedicine*, 4: 14.

Needleman SB, Wunsch CD 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. *Journal of Molecular Biology,* 48: 443-53.

Njoroge NG, Bussmann RW 2006. Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). *Journal of Ethnobiology and Ethnomedicine*, 108: 332-339.

Nomura N, Takaso T, Peng CI, Kono Y, Oginuma K, Mitsui Y, Setoguchi H 2010. Molecular phylogeny and habitat diversification of the genus Farfugium (Asteraceae) based on nuclear rDNA and plastid DNA. *Annals of Botany*, 106: 467-482.

Olila D, Olwa-Odyek B, Opuda-Asibo J 2001. Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. *African Health Sciences,* 1: 66-72.

Petit RJ, El Mousadik A, Pons O 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology,* 12: 844-855.

Qui YL, LiL Wang B, Chen Z, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff R, Stech M, Frey W, Quandt D, Davis CC 2006. The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences of the United States of America,* 103: 15511-15516.

Rabe T, van Staden J 2000. Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *Journal of Ethnopharmacology,* 73: 171-174.

Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, Tanahashi T, Sakakibara K, Fujita T, Oishi K, Shin-I T, Kuroki Y, Toyoda A, Suzuki Y, Hashimoto S, Yamaguchi K, Sugano S, Kohara Y, Fujiyama A, Anterola A, Aoki S, Ashton N, Barbazuk WB, Barker E, Bennetzen JL, Blankenship R, Cho SH, Dutcher SK, Estelle M, Fawcett JA, Gundlach H, Hanada K, Heyl A, Hicks KA, Hughes J, Lohr M, Mayer K, Melkozernov A, Murata T, Nelson DR, Pils B, Prigge M, Reiss B, Renner T, Rombauts S, Rushton PJ, Sanderfoot A, Schween G, Shiu SH, Stueber K, Theodoulou FL, Tu H, Van de Peer Y, Verrier PJ, Waters E, Wood A, Yang L, Cove D, Cuming AC, Hasebe M, Lucas S, Mishler BD, Reski R, Grigoriev IV, Quatrano RS, Boore JL 2008. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science*, 319: 64-69.

Rice PLI, Bleasby A 2000. "EMBOSS: The European Molecular Biology Open Software Suite". *Trends in Genetics*, 16: 276-277.

Sambrook J, Fritsch EF, Maniatis T 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, New York, USA: Cold Spring Harbor Laboratory Press.

Sasaki T 2006. Rice in deep water. *Nature*, 442:635-636.

Scholz CA, Cohen AS, Johnson TC, King J, Talbot MR, Brown ET 2011. Scientific drilling in the Great Rift Valley: The 2005 Lake Malawi Scientific Drilling Project – An overview of the past 145,000 years of climate variability in Southern Hemisphere East Africa. *Paleogeography, Paleoclimatology, Paleoecology,* 303: 3-19.

Sestras R, Doru P, Ardelean M, Botez C, Sestras A, Mitre I, Dan C, Mihalte L 2009. Use of phenotypic and MAS selection based on bulk segregant analysis to reveal the genetic variability induced by artificial hybridization in apple. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 37 (1): 273-277.

Slotkin R K, Martienssen R 2007. Transposable elements and the epigenetic regulation of the genome. *Nature Reviews Genetics,* 8: 272-285.

Sonnhammer ELL, Eddy SR, Birney E, Bateman A, Durbin R 1998. Pfam: Multiple sequence alignments and HMM-profiles of protein domains. *Nucleic Acids Research*, 26: 320-322.

Stecker C, Johann A, Herzberg C, Averhoff B, Gottschalk G 2003. Complete nucleotide sequence and genetic organization of the 210-kilobase linear plasmid of Rhodococcus erythropolis BD2, *Journal of Bacteriology*, 185: 5269-5274.

Tamura K, Dudley J, Nei M, Kumar S 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24: 1596-1599.

Thompson JD, Linard B, Lecompte O, Poch O 2011. A comprehensive benchmark study of multiple sequence alignment methods: Current challenges and future perspectives. *PLoS ONE*, 6(3): e18093. doi:10.1371/journal.pone.0018093.

Tulsiani SM, Craig SB, Graham GC, Cobbold RC, Dohnt MF, Burns MA, Leung LKP, Smythe LD 2010. High-resolution melt-curve analysis of random-amplified-polymorphic-DNA markers, for the characterisation of pathogenic Leptospira. *Annals of Tropical Medicine and Parasitology*, 104: 151-161.

Upadhyaya HD, Thudi M, Dronavalli N, Gujaria N, Singh S, Shivali S, Varshney RK 2011. Genomic tools and germplasm diversity for chickpea improvement. *Plant Genetic Resources: Characterization and Utilization*, 9: 45-58.

van Breugel P, Kindt R, Lillesø JPB, Bingham M, Demissew S, Dudley C, Friis I, Gachathi F, Kalema J, Mbago F, Minani V, Moshi HN, Mulumba J, Namaganda M, Ndangalasi HJ, Ruffo CK, Jamnadass R, Graudal L 2011. Potential Natural Vegetation of Eastern Africa. Volume 7. Projected Distributions of Potential Natural Vegetation Types and Two Important Agroforestry Species (*Prunus africanus* and *Warburgia ugandensis*) for Six Possible Future Climates. *Forest and Landscape Working Paper 69-2011*.

Watson J Geard N, Wiles J 2004. Towards more biological mutation operators in gene regulation studies. *Biosystems*, 76: 239-248.

Were PS, Kinyanju P, Gicheru MM, Mwangi E, Ozwara HS 2010. Prophylactic and curative activities of extracts from *Warburgia ugandensis* Sprague Canellaceae) and *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) against *Plasmodium knowlesi* and *Plasmodium berghei*. *Journal of Ethnopharmacology,* 130: 158-162.

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chaloub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH 2007. A unified classification system for eukaryotic transposable elements. *Nature Review Genetics,* 8: 973-982.

Williamson VM 1983. Transposable elements in yeast. *International Reviews in Cytology,* 83: 1-25.

Wube AA, Bucar F, Gibbons S, Asres K 2005. Sesquiterpenes from *Warburgia ugandensis* and their antimyco-bacterial activity. *Phytochemistry*, 66: 2309-2315.

Young A, Boyle T, Brown T 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution,* 11: 413-418.