

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 skim-

mianine 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; Sestras 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 μl PCR contained 1× PCR buffer, 2 mM MgCl₂, 200 μM of each dNTP, 0.13 μM of each primer. Table 2 shows the primers used in the study, 0.5 U *Taq*

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.

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 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 μl 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 μ g/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 μ l 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 μ l, ligated into 1 μ l pGEM-T easy vector (Promega) in *E. coli* (DH5 α) 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). Sequences 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 Genbank 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 Wunsch 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.ac.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-

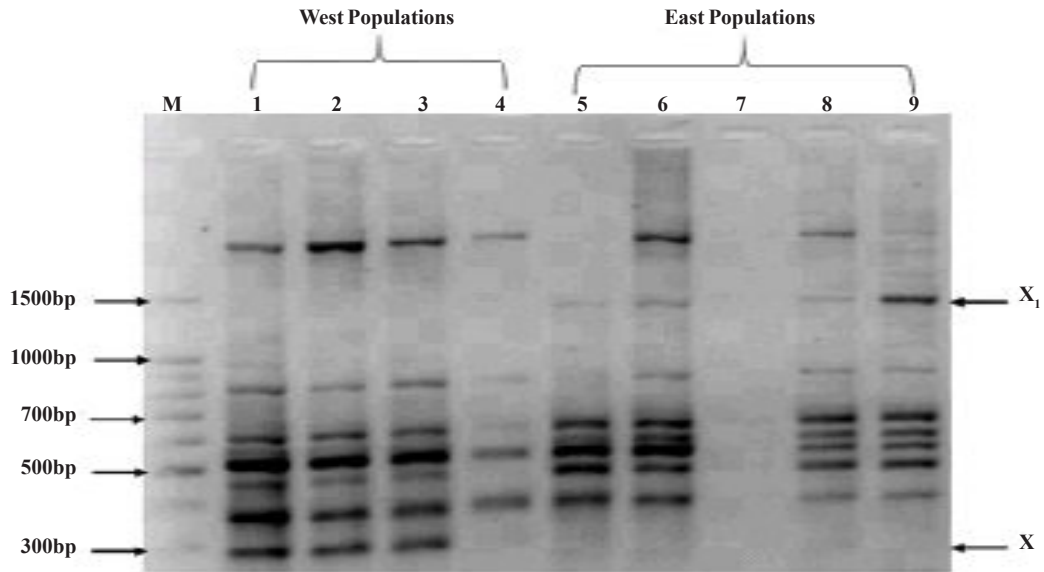


Fig. 2. RAPD profiles of *W. ugandensis* using primer IC21. X and X₁ arrows show RAPD bands revealing population differences, while M is a molecular size maker of 100 bps (Population 1 Kitale1 + Cherangani, 2 Kitale2 + Cherangani, 3 Kitale4+ Kibale, 4 Cherangani, 5 Londiani, 6 Karura1 + Laikipia, 7 Karura2 + Laikipia, 8 Karura1 + Taita, 9 Laikipia + Lushoto).

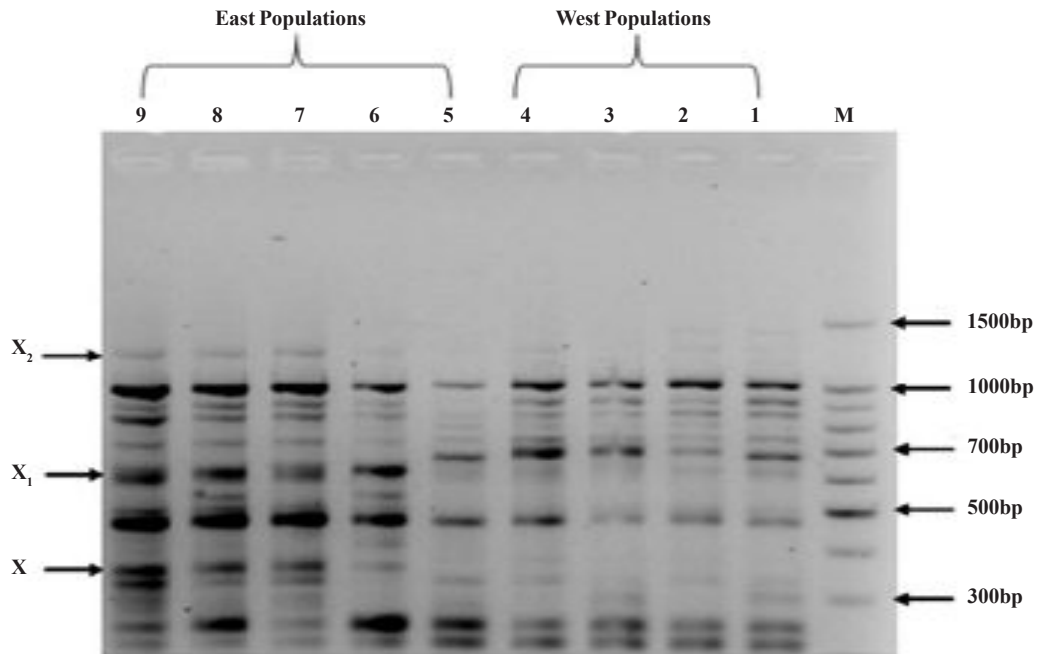


Fig. 3. RAPD profiles of *W. ugandensis* using primer IC28. X and X₁ arrows show RAPD bands revealing population differences, while M is a molecular size maker of 100 bps (Population 1 Kitale1 + Cherangani, 2 Kitale2 + Cherangani, 3 Kitale4+ Kibale, 4 Cherangani, 5 Londiani, 6 Karura1 + Laikipia, 7 Karura2 + Laikipia, 8 Karura1 + Taita, 9 Laikipia + Lushoto). X₂ 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
<i>Artemisia annua</i> strain <i>Artemis</i>	EZ153264.1	EZ153264	402 aa	1e-19	100%
<i>Collimonas fungivorans</i>	AY593480.1	AY593480	11502 aa	5e-16	90%
<i>Candidatus Glomeribacter gigasporarum</i>	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	<i>Warburgia</i>	264	2	<i>Artemisin</i>	402	43
1	<i>Warburgia</i>	264	3	<i>collimonas</i>	811	35
1	<i>Warburgia</i>	264	4	<i>Candidatus</i>	945	47
2	<i>Artemisin</i>	402	3	<i>collimonas</i>	811	51
2	<i>Artemisin</i>	402	4	<i>Candidatus</i>	945	56
3	<i>collimonas</i>	811	4	<i>Candidatus</i>	945	61

ids and their class. A reconstructed phylogenetic tree showed genetic distances and disparity in-

indices as shown on Figure 5 and explained in Table 5.

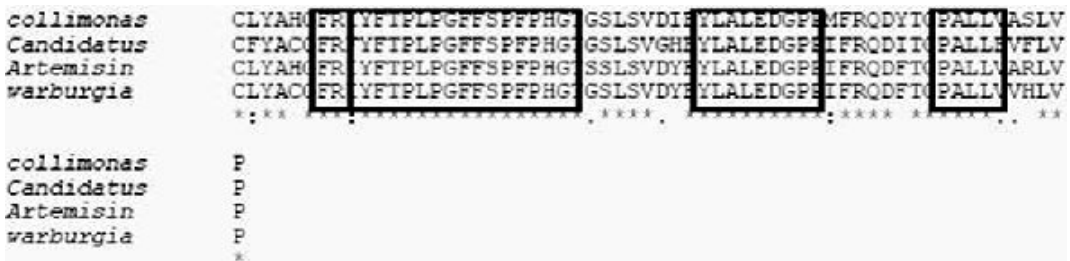


Fig.4. Alignments of the 3 *W. ugandensis* and significant retrieved homologous sequences in the study sequences submitted to NCBI accession numbers AY593480, AJ561042.3, EZ153264.1 in order as from above to *W. ugandensis* sequence homologs. (The symbols represents: (-) means many gaps, (“.”) very few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

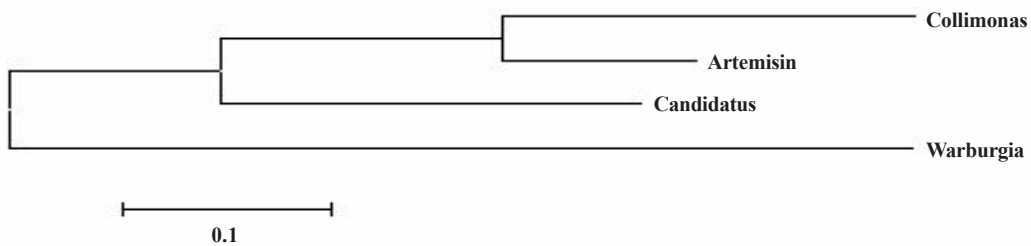


Fig. 5. Phylogram of the homologous sequences to all *W. ugandensis* significant sequences genetic pair-wise distance values of between 0.7 – 0.9

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

		1	2	3		1	2	3	4
1	<i>Collimonas</i>				<i>Collimonas</i>		(0.2)	(0.1)	(0)
2	<i>Artemisin</i>	0.5			<i>Artemisin</i>			(0)	(0)
3	<i>Candidatus</i>	0.3	0.4		<i>Candidatus</i>				(0)
4	<i>Warburgia</i>	0.9	0.7	0.8	<i>Warburgia</i>				

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 revealed 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*, *Artemisia 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 *Artemisia* 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 *Artemisia* showing a genetic distance and a disparity index between *Candidatus* and *Artemisia* 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 topographical 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 ge-

nome 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.

ACKNOWLEDGEMENTS

This work formed part of Ochieng' Noel Onyango's MSc. (Biotechnology) thesis at Kenyatta University, Nairobi. We gratefully acknowledge the assistance of Dr. Daniel Masiga of International Centre for Insect Physiology and Ecology (ICIPE) for cloning reagents and laboratory space support, Dr Rob Skilton of International Livestock Research Institute (ILRI) for reagents support, Dr. Francis McOdimba of Modern Medical Diagnostics Consultants and World Agroforestry Centre (ICRAF) entire staff support. Funding was provided by the World Agroforestry Centre (ICRAF) through Tree Genetics Resources and Domestication Unit (GRP1) and World Agroforestry Fellowship Awards.

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