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African Journal of Plant Science

Full Length Research Paper

A DNA-barcode for *Melia volkensii* Gürke (Meliaceae) and its phylogenetic relationship with some economically important relatives

Eliud S. Mulanda[1]*, Ryan M. Awori[1], Yeremiah Chuhila[2], Mark O. Adero[1], Nelson O. Amugune[1], Elijah Akunda[1] and Jenesio I. Kinyamario[1]

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The study reports the first DNA-barcode and molecular phylogeny of the East African endemic tree species *Melia volkensii* using the standard two-locus plant barcoding genes (*rbc*L and *matK*). The two genes were amplified and the PCR products sequenced. Complete coding sequences were obtained for both genes. The edited and aligned sequences had lengths of 1371 bp for *rbc*L and 1524 bp for *mat*K. These DNA sequences were deposited into the DNA Data Bank of Japan (DDBJ) with cross-listing in the European Molecular Biology Labaratory (EMBL) and GenBank databases. The deposited gene sequences were then subjected to separate nucleotide BLASTs in NCBI's GenBank database. Out of 100 Blast results in which the query (*M. volkensii*) had 96–100 percentage similarity in nucleotide sequence for the *rbc*L gene and 90-100% similarity for the *mat*K gene, only 16 taxa had data for both *rbc*L and *mat*K genes. These 16 taxa were used for the phylogenetic analysis and comprised of 6, 9 and 1 taxa respectively from the families Meliaceae, Simaroubaceae and Rutaceae. The barcode allowed adequate discrimination of the taxa into their respective generic and species clades. Availability of a barcode for *M. volkensii* will ease identification of the species, provide more robust phylogenetic reconstructions and allow for better tracking of its exotic dispersal.

Key words: DNA barcoding, *matK*, *rbcL*, DDBJ/EMBL/NCBI Gene Databases, *Melia volkensii,* phylogeny.

INTRODUCTION

Melia volkensii (Gurke) is a hardwood tree species of high economic, ecological and germplasm value. It is endemic to the arid and semi-arid lands of East Africa and belongs to the mahogany family, Meliaceae (Orwa et al., 2009). Other members of the family known for their significant timber, pharmaceutical and conservation value

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are *Azadirachta indica* A. Juss. (Neem), *Melia azedarach* L. (Purple Lilac), *Swietenia macrophylla* (Big-leaf mahogany) and the *Khaya* species.

The primary objective of the study was to develop a DNA barcode sequence for *M. volkensii*. DNA barcoding is the use of nucleotide diversity within a short standardised region of DNA for identification of species (Hebert et al., 2003; Kuzmina et al., 2012; Vijayan and Tsou, 2015). DNA barcoding provides an automated species identification system that is quicker and more reliable than traditional taxonomic methods which rely on morphological characters (Newmaster and Ragupathy, 2009). DNA barcodes can not only resolve phylogenies of plant taxa but are also useful in ecological forensics such as the tracking of illegal trade in plant products (Kress et al., 2015). Other applications of a DNA barcode include monitoring of exotic dispersion, conservation impact assessments, authentication of parts used in preparation of herbal medicine and botanical pesticides, such as tree barks, fruits and leaves (Ferri et al., 2008; 2015; Kritpetcharat et al., 2011; Mankga et al., 2013; Mishra et al., 2016).

Until recently, DNA barcoding of plants was hampered by the lack of a standard region of DNA with sufficient universality, sequence quality and species discrimination power (Hollingsworth et al., 2011). The long search for a universal plant barcode culminated in the adoption of a two-locus barcode consisting of the phylogenetically conserved gene for the large subunit of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/ oxygenase (rubisco), also known as *rbc*L, and the more rapidly evolving chloroplast gene for maturase K (*matk)* (Kress et al., 2009). The 2-locus combination of *rbc*L and *mat*K genes was adopted by the Consortium for the Barcode of Life Plant Working Group (CBOL, 2009) as the standard or core barcode for land plants.

The *rbc*L gene is a chloroplast gene of approximately 1400 bp that codes for the large subunit of rubisco, the enzyme that catalyzes carbon dioxide fixation in chloroplasts. The *mat*K gene, approximately 1500 bp, is located within a 2,400 bp group II intron of the chloroplast *trn*K gene which codes for the transfer RNA for lysine (Johnson and Soltis, 1994; Vogel et al., 1997; Steane, 2005; Hausner et al., 2006; Barthet and Hilu, 2007). It codes for maturase K, an enzymatic protein that allows the intron to remove itself for the two exons of the *trn*K gene to be spliced together.

A secondary objective of the study was to use the novel barcode sequences in a preliminary phylogenetic study of the Meliaceae and related families. A molecular phylogeny based on DNA barcoding could clarify evolutionary relationships between both the well-known and lesser known members of the family.

This study reports the first DNA barcode for *Melia volkensii*. The availability of such a barcode for the species is will enable faster and accurate identification of the species and a more robust reconstruction of phylogenetic relationships in the family. This will provide insights on the phylogenetic affinities between *M. volkensii,* well-known members of the family such as *A. indica*, *M. azederach* and *S. macrophylla* and the lesser known ones. Phylogenetic affinities at the family and generic levels could also reveal closely related families and genera for novel bio-prospecting for compounds of pharmaceutical and pesticidal importance similar to those found in some members of the Meliaceae.

MATERIALS AND METHODS

Plant materials and DNA extraction

DNA was extracted from shoot tips of 20 *M. volkensii* seedlings obtained from seeds collected from Mavuria provenance in Mbeere, Embu county, Eastern Kenya (Geo-reference 0° 46.379'S, 37° 39.308'E). DNA Extraction was done using the Cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1987), with slight modifications, which were addition of 10% sodium dodecyl sulphate to extraction buffer, centrifugation at 16,000g instead of 6,000g and washing of the DNA pellet with 70% ethanol instead of a mixture of 76% ethanol and 10mM ammonium acetate.

Molecular methods

M. volkensii complete coding sequence for ribulose-1,5-carboxylase/oxygenase (rubisco) large subunit chloroplast gene (*rbcL*) was amplified by the PCR method. The expected fragment size was 1397bp (Fazekas et al., 2012). The primers used for *rbc*L gene were *rbclFayf* (5'TCCTTTTAGTAAAAGATTGGGCCGAG3') and *rbclFayr* (5'ATGTCACCACAAACAGAAACTAAAGC3') (Fay et al*.,* 1998). Primers were synthesised by Inqaba Biotec, South Africa. The reaction mixture contained 1 unit of MyTaq® DNA polymerase (Bioline, USA), 1x Mytaq buffer® (Bioline, USA) containing 3 mM $MgCl_2$ and 2 mM dNTPs; 0.4 µM forward and reverse primers, 1 µl of DNA template and brought to the total volume of 25 µl with nuclease-free water. Amplification was done on a MJ Research PTC-100 USA thermal cycler with the following conditions; initial denaturation at 95°C for 1 min , 40 cycles of at 95°C for 15 s (denaturation), 55°C for 15 s (annealing), 72°C for 1 min 30 s (extension), and a final extension at 72°C for 7 min.

Isolation of *M. volkensii* maturase-K chloroplast gene (*matK*) was also carried out in a 25 µl volume reaction. The expected fragment size was 1500 bp (Fazekas et al., 2012). The primers used were *Matk1f* (5'ACTGTATCGCACTATGTATCA3') and *Matk1r* (5'GAACTAGTCGGATGGAGTAG3'), also sourced from Inqaba Biotec South Africa. The reaction mixture contained 1 unit of MyTaq® DNA polymerase (Bioline, USA); 1x Mytaq® buffer (Bioline, USA) containing 3mM $MgCl_2$ and 2 mM dNTPs; 0.4 µM of forward and reverse primers, 1 µl of DNA template and brought to the total volume of 25 µl with nuclease-free water. Amplification was done on a MJ Research PTC-100 USA thermal cycler with conditions set at 95°C for 1 min, 20 cycles of 95°C for 15 s, 45°C for 15 s, 72°C for 1.5 min, followed by another 20 of cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 1.5 min and a final extension at 72°C for 5 min.

PCR products were purified with EXO/SAP Amplicon purification kit (Affymetrix, Santa Clara, USA). Purified PCR products were sequenced by Inqaba Biotec South Africa using The BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems,USA) with ABI Prism 377 DNA sequencer (Applied Biosystems,USA). The same primers used for the PCR reactions were used in sequencing

reactions.

Database deposition and phylogenetic reconstruction

*M. volkensii rbc*L and *mat*K novel sequences were checked for quality and ambiguous nucleotides resolved in MEGA6 software suite (Tamura et al., 2013). Identical sequences were obtained for each gene. Processed sequences of the two genes were deposited in the DDBJ/EMBL/GenBank databases. They were assigned the following accession numbers: LC075516 for *rbc*L and LC075517 for *mat*K.

The sequences were then used to carry out two separate GeneBank nucleotide BLASTs. The first set of 100 Blast hits gave 96–100 percentage similarity in nucleotide sequence for the rbcL gene and 90-100% similarity for the matK gene between the query (*M. volkensii*) and the respective Genbank sequences of members of Meliaceae, Simaroubaceae and Rutaceae families. However, retrieved taxa having sequence data for both rbcL and matK genes were only 16, with the rest of the taxa having data for either rbcL or matK. Since the study intended to use both the barcoding genes separately and after concatenation, phylogenetic reconstruction was limited to the sequences of these 16 taxa. Sequence names, database codes, accession numbers, native distribution and uses of the selected species are listed in Table 1.

The retrieved database sequences were also checked for quality and ambiguous nucleotides resolved in MEGA6 software suite (Tamura et al., 2013). Multiple sequence alignments were performed in MEGA6 software suite using the MUSCLE algorithm (Edgar, 2004) and the aligned sequences used for phylogenetic reconstruction. The evolutionary history was inferred using the maximum likelihood method based on the General Time Reversible (GTR) model (Nei and Kumar, 2000). Initial trees for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pair-wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree with the highest log likelihood was selected. A total of 1000 bootstrap replicates were performed (Felsenstein, 1985). Phylogenetic trees were edited in FigTree 1.4 (Rambaut, 2012).

RESULTS AND DISCUSSION

PCR amplification was 100% successful for both genes. Gel electrophoresis gave highly resolved bands of ≈ 1400 bp for *rbcL* and ≈ 1500 bp for *matK*, as expected (Figure 1). Sequencing success was 95% for both genes, with edited sequence lengths of 1371 bp for the *rbc*L gene and 1524 bp for *mat*K. These sequences were successfully deposited in the DDBJ/EMBL/GenBank databases and assigned the accession numbers LC075516 (*rbc*L) and LC075517 (*mat*K). To the best of our knowledge, they are the first barcode deposits for *M. volkensii* in these databases.

The BLASTs retrieved taxa belonging to three families: Meliaceae, Simaroubaceae and Rutaceae. This is in agreement with previous reports about the taxonomic proximity of these families (Wiart, 2006). However, most of the taxa had sequence data for either *rbc*L or *mat*K but not both. Therefore analysis was limited to the 16 closely related taxa which had sequences for both the *rbc*L and *mat*K genes. These consisted of 6 members of the Meliaceae family, 9 members of Simaroubaceae and 1 member of Rutaceae (Table 1). Consequently, phylogenetic reconstruction was severely constrained by the limited nature of the data retrieved from the databases. A more comprehensive molecular phylogeny of the Meliaceae will be possible only when more sequence data becomes available in these databases. Since the family Meliaceae consists of an estimated 51 genera and 550 species (Wiart, 2006), there is a vast scope for an expanded molecular phylogeny of the family.

The taxa included in the phylogenetic analysis had sequence percentage alignment scores of 96-99% for *rbc*L gene and 90-95% for the *mat*K gene (Table 1). This is in agreement with previous reports of higher discrimination power of *mat*K over *rbc*L for most plants (Li et al., 2011). This difference was also evident in the pair-wise distance matrices (Tables 2 and 3) and phylogenetic trees (Figures 2 and 3), with *mat*K giving larger genetic distances between the species than *rbc*L and the concatenated *rbcL* + *mat*K code giving intermediate distances (Table 4). This was expected as the *mat*K gene is reported to have a higher rate of mutation than the *rbc*L gene (Kress et al., 2009) and is thus more likely to reveal a greater amount of variation between species. The *rbc*L locus is generally more suitable for determination of evolutionary relationships at the generic level and above (Kress et al., 2005). On the other hand *mat*K has been more successful in resolving species relationships in several families (Johnson and Soltis, 1994; Hilu and Liang, 1997; Rohwer, 2000).

All the phylogenetic trees obtained with separate *rbcL*, *matK* sequences and with concatenated *rbcL* + *matK* sequences correctly resolved the 17 taxa into their respective familial clades with 100% bootstrap support (Figures 2, 3 and 4). In each family, the vast majority of branches also had high bootstrap values (> 90%). These barcoding genes also allowed adequate discrimination at generic and species levels, as seen in the clear resolution of the genus *Melia* (*M. volkensii* and *M. azederach*), genus *Swietenia* (*S. macrophylla* and *S. mahogany*), genus *Picrasma* (*P. javanica and P. quassioides*) and genus *Ailanthus* (*A. integrifolia*, *A. altissima* and *A. triphysa*). This suggests a possible use of the two barcoding genes, with additional empirical testing, in resolving taxa in the Meliaceae and related families up to the species level. This recommendation is supported by the findings of Kress et al. (2005) which showed that full-length sequences (>1 kb) of either gene can give enough sequence length to discriminate between species. The sequences obtained in this study were longer than 1kb and therefore met this criterion.

Despite the limited number of taxa used, the molecular phylogeny obtained in this study provides some useful insights into the evolutionary relationships between *M. volkensii* and the taxa that were included in the phylogeny. This is one of the suggested applications of a DNA barcode (Kress et al., 2015). The *M. volkensii*

Table 1. Species information and nucleotide BLAST alignment scores for *Melia volkensii* (DDBJ LC075516.1 and LC075517.1) and selected species.

Family	Species name (Common name)	Native distribution	Main uses	Similarity With *M. volkensii* (%)		Database/ Accession number	
				*rbc*L	*mat*K	*rbc*L	*mat*K
Meliaceae	*Melia azederach* L. (Purple lilac)	Indian subcontinent and South East Asia	Timber, medicinal, insecticidal, ornamental	99	99	GB/AY128234.1	GB/EF489117.1
	Azadirachta indica A. Juss. (Neem)	Indian subcontinent and South East Asia	Timber, medicinal, insecticidal, ornamental	99	97	GB/AY128214.1	GB/EF489115.1
	Toona sinensis (A.Juss.) M. Roem (Chinese mahogany)	Eastern and South Eastern Asia	Timber, medicinal, ornamental	97	94	EMB/FN599468.1	GB/JN680341.1
	Swietenia macrophylla King. (Honduran mahogany)	Mexico and South America	Timber, medicinal, ornamental	97	93	GB/U39080.2	GB/EF489114.1
	Swietenia mahogany (L.) Jacq. (West Indies mahogany)	Caribbean Islands and USA	Timber, medicinal, ornamental	97	93	EMB/FN599465.1	GB/EU042835.1
	Cipadessa baccifera (Roth) Miq.	India, Sri Lanka, Myanmar, China, Malaysia	Medicinal	96	94	GB/AY128225.1	GB/EF489116.1
Simaroubaceae	*Ailanthus integrifolia* Lam. (White Siris)	India, Indonesia, Malaysia, Papua New Guinea	Timber, Medicinal	96	91	GB/EU042981.2	GB/042843.1
	Ailanthus altissima (Mill. Swingle; (Tree of Heaven)	China and Taiwan	Timber, Medicinal, Ornamental	96	91	GB/KM360619.1	EMB/FM179922.1
	Ailanthus triphysa (Dennst.) Alston (White Siris)	India, Myanmar, Nepal	Timber, Medicinal, Ornamental	96	91	GB/EU042982.1	GB/EU042844.1
	Castela retusa Liebm.;	Mexico and Central America	Medicinal	96	90	GB/EU042992.1	GB/EU042854.1
	Picrasma quassioides (D,Don) Benn. (Quassia wood)	Eastern and South America; East Asia	Timber, Medicinal, Insecticidal	96	91	GB/EU043008.1	GB/EU042870.1
	Picrasma javanica Blume	India, Bangladesh, Java, Burma, Malaysia	Timber, Medicinal	96	91	GB/EU043011.1	GB/EU042873.1
	Nothospondias staudtii Engl.	West Africa and the DR Congo	Timber, Medicinal	96	91	GB/EU043004.1	GB/EU042866.1
	Holocantha emoryi A. Gray	South western USA	Medicinal	96	91	GB/EU043002.1	GB/EU042864.1
	Hannoa klaineana Pierre and Engl.	West and Central Africa	Timber, Medicinal	96	90	GB/EU042999.1	GB/EU042861.1
Rutaceae	*Choisya ternata* Kunth (Mexican Orange)	Mexico	Ornamental, Medicinal	96	91	GB/KM360716.1	GB/EF489104.1

barcode could also be useful in aiding identification of the species and its products, enabling more detailed phylogenetic reconstructions and the tracking of its exotic dispersion. However, for application of the *mat*K + *rbc*Lplant barcode in a more comprehensive study of the Meliaceae, there is an urgent need for sequencing of the *rbc*L and *mat*K genes for all the estimated 550 species of the Meliaceae and deposition of the data

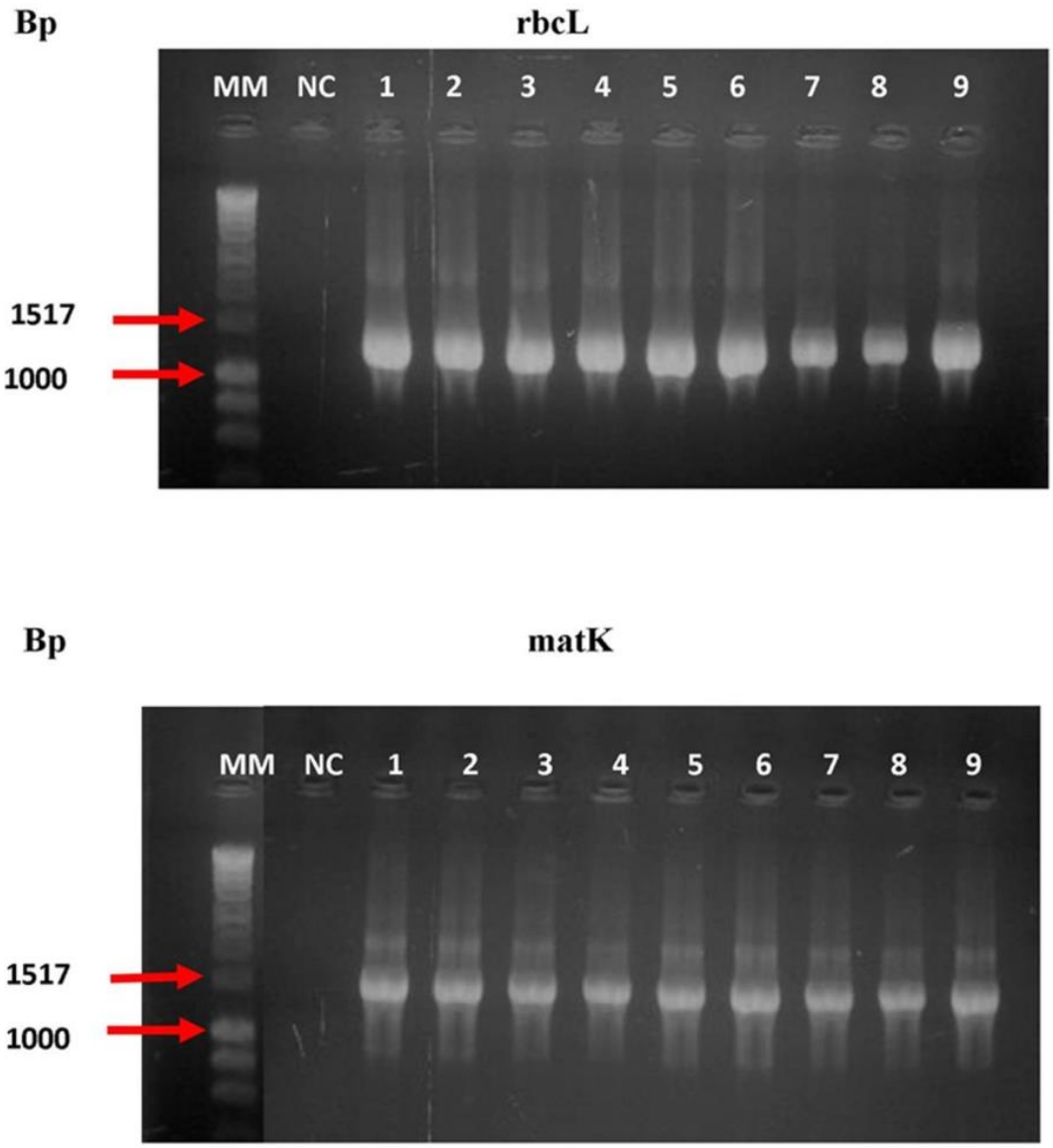

Figure 1. Agarose gel profiles of the isolated chloroplast *rbc*L and *mat*K genes. MM= 1kb ladder, NC= negative control, 1-9 = some of the DNA samples used.

in DNA Databases.

Conclusions and recommendations

The plant barcoding genes *rbc*L and *mat*K managed to resolve selected taxa up to the species level. A partial molecular phylogeny of the Meliaceae and closely related famlilies was obtained. The main limiting factor was the lack of complete data on *rbc*L and *mat*K sequences in the DNA repositories for members of these families. This calls for accelerated deposition of more sequence data in order to fill the huge gaps in the DNA libraries. Such data can also be used in future Bayesian inferences.

Conflict of interest

The authors have not declared any conflict of interests.

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Table 2. Estimates of genetic distance between sequences using *rbc*L alone, based on the number of base substitutions per site. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		Rubisco (*rbc*L)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Melia_volkensii*_{Meliaceae}		0.002	0.003	0.005	0.005	0.005	0.005	0.005	0.006	0.005	0.006	0.006	0.005	0.006	0.006	0.006	0.006
2	*Melia_azedarach*_{Meliaceae}	0.004		0.003	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.005
3	*Azadirachta_indica*_{Meliaceae}	0.013	0.012		0.004	0.004	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.006	0.005	0.006	0.005
4	*Swietenia_macrophylla*_{Meliaceae}	0.029	0.028	0.024		0.002	0.001	0.004	0.004	0.005	0.004	0.005	0.005	0.004	0.005	0.005	0.006	0.005
5	*Toona_sinensis*_{Meliaceae}	0.026	0.027	0.023	0.007		0.002	0.004	0.004	0.005	0.005	0.004	0.005	0.004	0.005	0.005	0.005	0.005
6	*Swietenia_mahagoni*_{Meliaceae}	0.029	0.030	0.027	0.003	0.006		0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
7	*Picrasma_quassioides*_{Simaroubaceae}	0.037	0.037	0.034	0.027	0.025	0.027		0.001	0.004	0.005	0.004	0.004	0.004	0.004	0.004	0.006	0.004
8	*Picrasma_javanica*_{Simaroubaceae}	0.037	0.038	0.034	0.026	0.024	0.027	0.003		0.004	0.005	0.004	0.004	0.004	0.004	0.004	0.006	0.004
9	*Castela_retusa*_{Simaroubaceae}	0.039	0.040	0.040	0.032	0.030	0.032	0.022	0.023		0.006	0.005	0.005	0.005	0.003	0.005	0.007	0.005
10	*Choisya_ternata*_{Rutaceae}	0.041	0.040	0.038	0.028	0.030	0.031	0.034	0.034	0.039		0.005	0.005	0.005	0.006	0.005	0.006	0.005
11	*Ailanthus_integrifolia*_{Simaroubaceae}	0.040	0.040	0.037	0.031	0.030	0.032	0.023	0.023	0.027	0.037		0.002	0.003	0.005	0.002	0.006	0.004
12	*Ailanthus_altissima*_{Simaroubaceae}	0.040	0.041	0.038	0.033	0.031	0.034	0.023	0.024	0.029	0.038	0.004		0.003	0.005	0.002	0.006	0.004
13	*Nothospondias_staudtii*_{Simaroubaceae}	0.041	0.040	0.035	0.030	0.030	0.033	0.023	0.025	0.028	0.039	0.017	0.017		0.005	0.004	0.006	0.003
14	*Holacantha_emoryi*_{Simaroubaceae}	0.042	0.040	0.040	0.033	0.032	0.034	0.024	0.027	0.009	0.041	0.029	0.028	0.028		0.005	0.007	0.005
15	*Ailanthus_triphysa*_{Simaroubaceae}	0.042	0.043	0.040	0.034	0.033	0.034	0.024	0.026	0.030	0.039	0.004	0.006	0.019	0.030		0.007	0.004
16	*Cipadessa_baccifera*_{Meliaceae}	0.043	0.044	0.039	0.037	0.036	0.038	0.045	0.043	0.049	0.045	0.052	0.054	0.051	0.052	0.056		0.006
17	*Hannoa_klaineana*_{Simaroubaceae}	0.047	0.047	0.043	0.036	0.034	0.037	0.028	0.030	0.033	0.041	0.020	0.020	0.017	0.034	0.020	0.053	

Table 3. Estimates of genetic distance between sequences using *mat*K alone, based on the number of base substitutions per site. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		Maturase K (*mat*K)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Melia_volkensii*_{Meliaceae}		0.003	0.005	0.008	0.009	0.009	0.009	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012
2	*Melia_azedarach*_{Meliaceae}	0.015		0.005	0.007	0.009	0.009	0.009	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012
3	*Azadirachta_indica*_{Meliaceae}	0.035	0.028		0.008	0.008	0.009	0.009	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.011	0.012
4	*Cipadessa_baccifera*_{Meliaceae}	0.060	0.054	0.055		0.007	0.008	0.008	0.009	0.009	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011
5	*Toona_sinensis*_{Meliaceae}	0.064	0.062	0.059	0.046		0.003	0.003	0.009	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011
6	*Swietenia_mahogani*_{Meliaceae}	0.067	0.064	0.062	0.051	0.010		0.001	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011
7	*Swietenia_macrophylla*_{Meliaceae}	0.069	0.065	0.063	0.052	0.011	0.003		0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011

Table 3. Contd.

8	*Picrasma_javanica*_{Simaroubaceae}	0.087	0.082	0.082	0.069	0.072	0.074	0.075		0.002	0.007	0.007	0.007	0.006	0.007	0.009	0.008	0.008
9	*Picrasma_quassioides*_{Simaroubaceae}	0.088	0.083	0.083	0.070	0.072	0.075	0.075	0.004		0.007	0.007	0.007	0.007	0.008	0.009	0.008	0.008
10	*Ailanthus_triphysa*_{Simaroubaceae}	0.091	0.087	0.087	0.078	0.076	0.077	0.078	0.044	0.046		0.004	0.008	0.004	0.006	0.010	0.009	0.006
11	*Ailanthus_integrifolia*_{Simaroubaceae}	0.094	0.090	0.090	0.080	0.076	0.078	0.079	0.044	0.047	0.017		0.008	0.004	0.006	0.010	0.009	0.006
12	*Holacantha_emoryi*_{Simaroubaceae}	0.094	0.088	0.090	0.083	0.082	0.086	0.085	0.051	0.052	0.061	0.059		0.008	0.008	0.010	0.004	0.009
13	*Ailanthus_altissima*_{Simaroubaceae}	0.094	0.090	0.089	0.078	0.074	0.076	0.076	0.044	0.046	0.017	0.017	0.058		0.006	0.010	0.009	0.006
14	*Nothospondias_staudtii*_{Simaroubaceae}	0.094	0.091	0.092	0.080	0.078	0.082	0.083	0.051	0.051	0.035	0.035	0.059	0.036		0.010	0.008	0.006
15	*Choisya_ternata*_{Rutaceae}	0.094	0.087	0.089	0.081	0.077	0.081	0.082	0.072	0.071	0.079	0.080	0.082	0.080	0.080		0.011	0.011
16	*Castela_retusa*_{Simaroubaceae}	0.100	0.094	0.091	0.091	0.090	0.093	0.094	0.057	0.058	0.065	0.065	0.022	0.066	0.064	0.090		0.010
17	*Hannoa_klaineana*_{Simaroubaceae}	0.102	0.098	0.099	0.089	0.084	0.086	0.087	0.053	0.055	0.037	0.035	0.067	0.038	0.037	0.087	0.074	

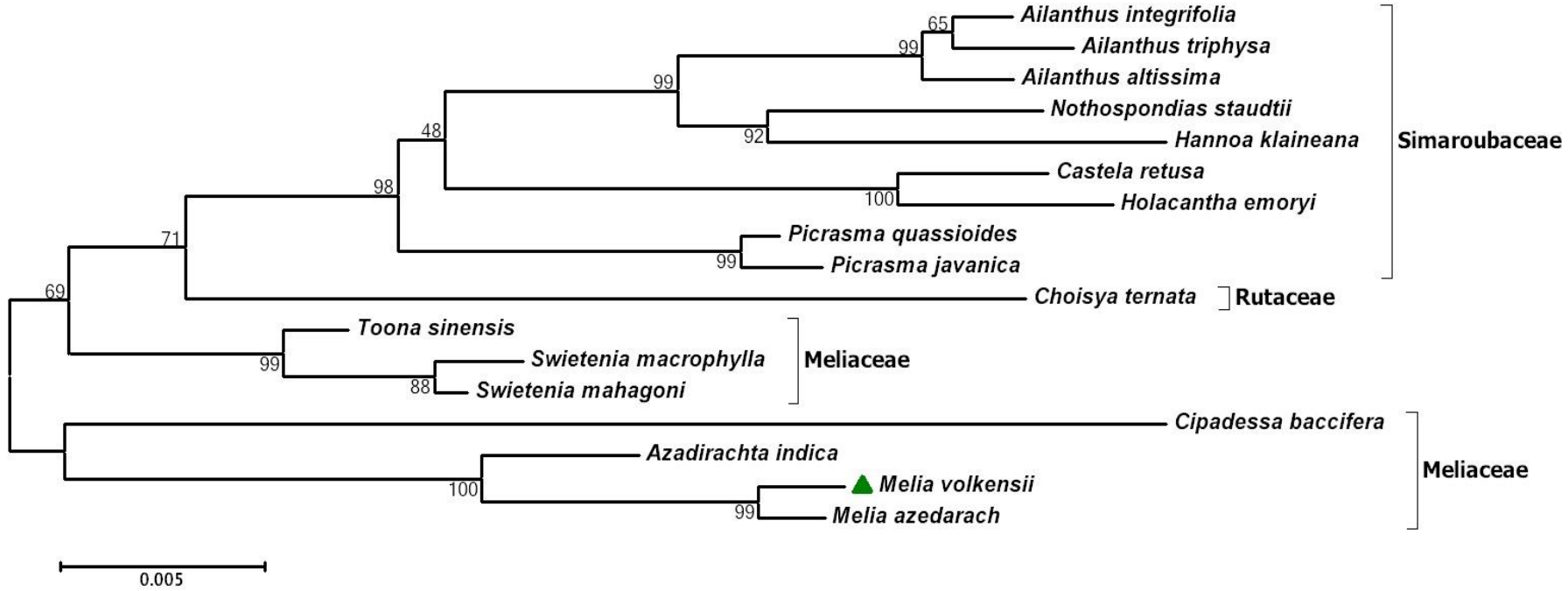

Figure 2. Maximum Likelihood phylogenetic tree for *Melia volkensii* and 16 closely related species based on *rbc*L gene, with 1000 bootstraps. Bootstrap support values are shown at nodes. Scale = number of substitutions per site.

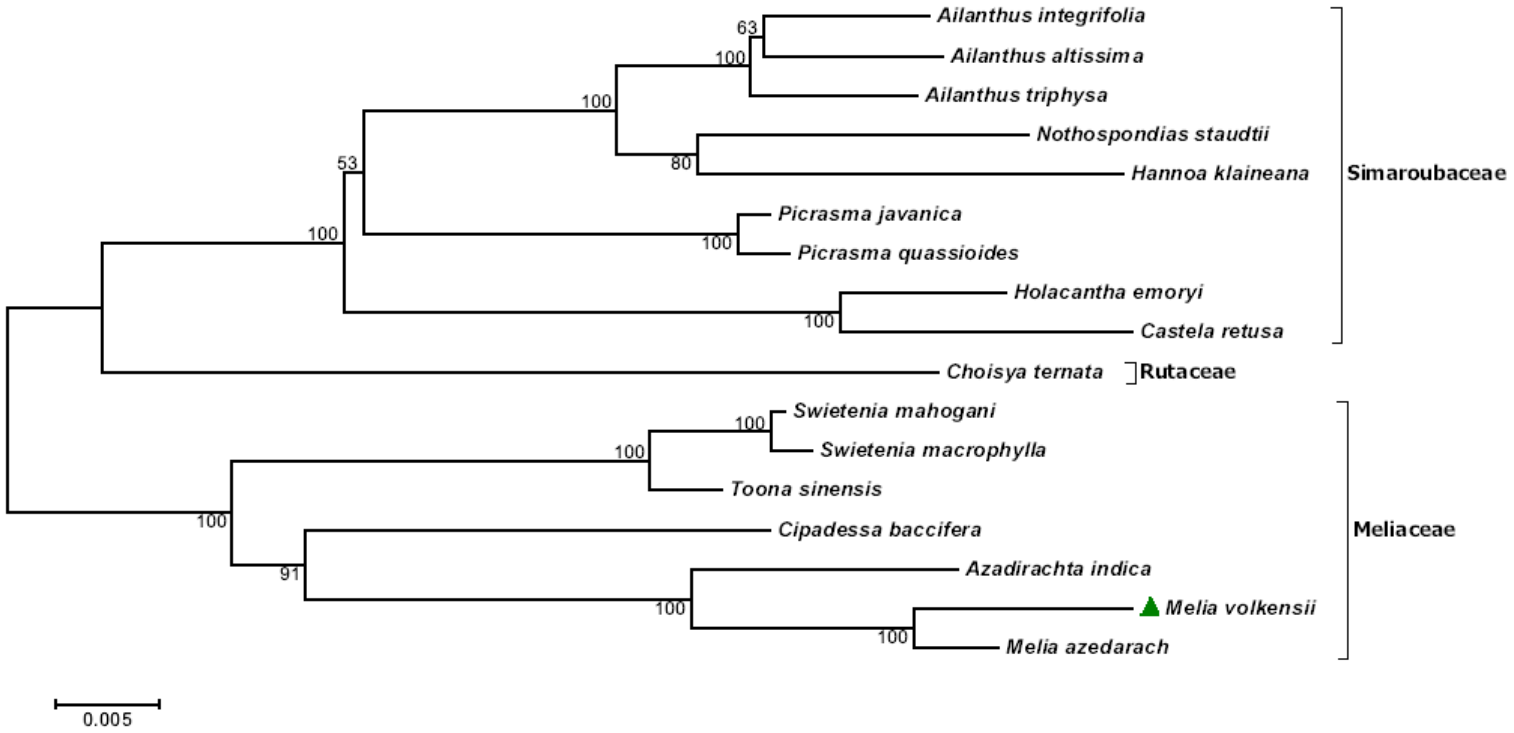

Figure 3. Maximum Likelihood phylogenetic tree for *Melia volkensii* and 16 closely related species based on *mat*K gene, with 1000 bootstraps. Bootstrap support values are shown at nodes. Scale = number of substitutions per site.

Table 4. Estimates of genetic distance between sequences using *rbc*L + *mat*K concatenated sequences, based on the number of base substitutions per site. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates).

		***rbc*L + *mat*K concatenated**																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Melia_volkensii*_{Meliaceae}		0.002	0.003	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
2	*Melia_azedarach*_{Meliaceae}	0.010		0.002	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
3	*Azadirachta_indica*_{Meliaceae}	0.024	0.020		0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
4	*Cipadessa_baccifera*_{Meliaceae}	0.052	0.049	0.047		0.004	0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
5	*Toona_sinensis*_{Meliaceae}	0.046	0.045	0.042	0.041		0.002	0.002	0.004	0.004	0.005	0.004	0.005	0.004	0.004	0.004	0.005	0.004
6	*Swietenia_mahogani*_{Meliaceae}	0.049	0.048	0.045	0.045	0.008		0.001	0.005	0.004	0.005	0.004	0.005	0.004	0.005	0.004	0.005	0.004

Table 4. Contd.

7	*Swietenia_macrophylla*_{Meliaceae}	0.050	0.047	0.044	0.045	0.010	0.003		0.005	0.005	0.005	0.004	0.005	0.005	0.004	0.004	0.005	0.004
8	*Picrasma_javanica*_{Simaroubaceae}	0.063	0.061	0.059	0.056	0.049	0.051	0.051		0.001	0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.004
9	*Picrasma_quassioides*_{Simaroubaceae}	0.063	0.061	0.059	0.058	0.050	0.052	0.052	0.004		0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.004
10	*Ailanthus_triphysa*_{Simaroubaceae}	0.068	0.066	0.064	0.067	0.055	0.057	0.057	0.035	0.036		0.002	0.004	0.002	0.003	0.004	0.004	0.003
11	*Ailanthus_integrifolia*_{Simaroubaceae}	0.068	0.066	0.065	0.066	0.054	0.056	0.056	0.034	0.035	0.011		0.004	0.002	0.003	0.004	0.004	0.003
12	*Holacantha_emoryi*_{Simaroubaceae}	0.069	0.065	0.066	0.068	0.058	0.061	0.060	0.040	0.039	0.046	0.044		0.004	0.004	0.005	0.003	0.004
13	*Ailanthus_altissima*_{Simaroubaceae}	0.068	0.066	0.065	0.066	0.053	0.055	0.055	0.034	0.035	0.012	0.011	0.044		0.003	0.005	0.004	0.003
14	*Nothospondias_staudtii*_{Simaroubaceae}	0.068	0.066	0.064	0.066	0.055	0.058	0.058	0.038	0.038	0.027	0.027	0.044	0.027		0.004	0.004	0.003
15	*Choisya_ternata*_{Rutaceae}	0.069	0.064	0.065	0.063	0.054	0.057	0.056	0.054	0.053	0.060	0.059	0.062	0.060	0.060		0.005	0.004
16	*Castela_retusa*_{Simaroubaceae}	0.070	0.068	0.066	0.070	0.061	0.064	0.064	0.041	0.041	0.049	0.047	0.016	0.048	0.047	0.066		0.004
17	*Hannoa_klaineana*_{Simaroubaceae}	0.075	0.073	0.071	0.072	0.060	0.062	0.062	0.041	0.042	0.029	0.028	0.051	0.029	0.027	0.065	0.054	

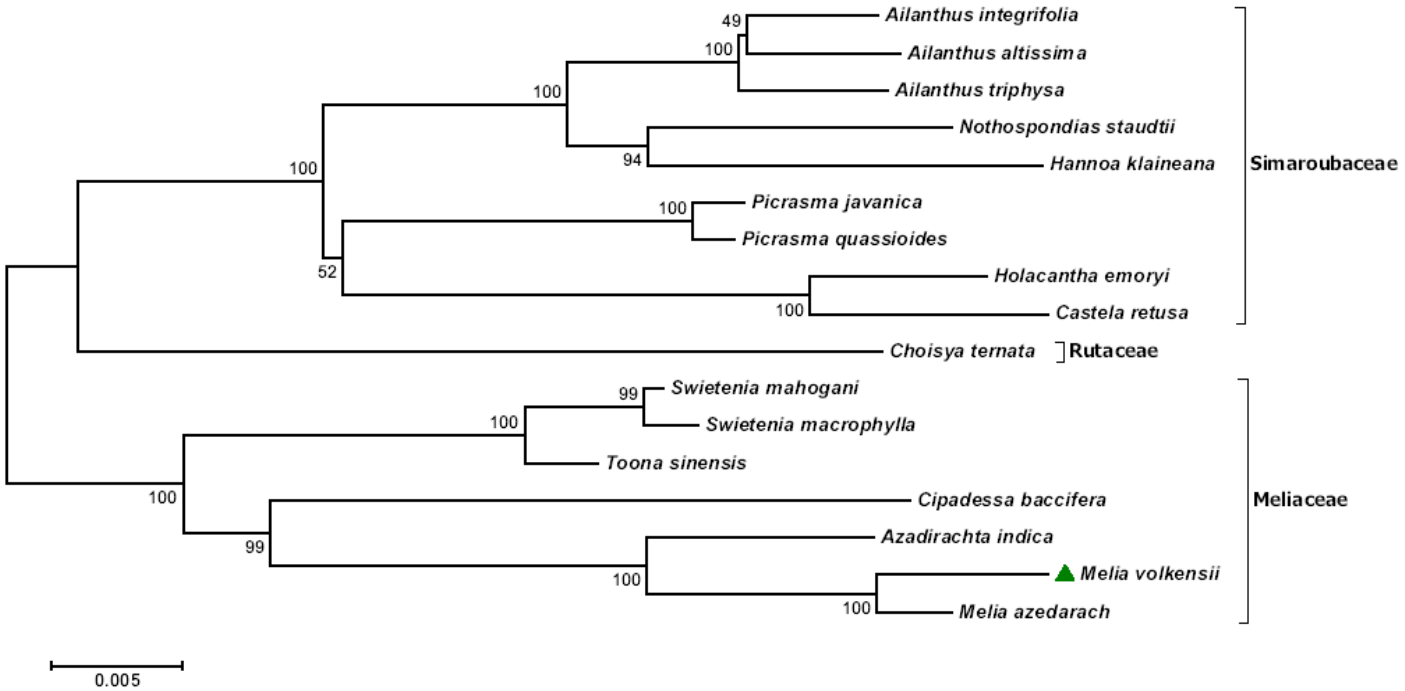

Figure 4. Maximum Likelihood phylogenetic tree for *Melia volkensii* and 16 closely related species based on *rbc*L + *mat*K concatenated genes, with 1000 bootstraps. Bootstrap support values are shown at nodes. Scale = number of substitutions per site.

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Full Length Research Paper

Growth responses of two varieties of Heliconia flowers to selected growth media in Port Harcourt, South-South Nigeria

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Lack of suitable variety as well as appropriate growth medium constitutes major problems to flower production. It is against this backdrop that a pot experiment was carried out in the Department of Crop and Soil Science Demonstration Plot, Faculty of Agriculture, University of Port Harcourt, Nigeria between April and May, 2013 to determine the growth responses of two varieties of Heliconia flowers to selected growth media. The experiment comprised of two Heliconia varieties (Jade and Golden Torch) and six (6) growth media namely, topsoil (control), river sand, coconut husk, topsoil + river sand (1:1), topsoil + coconut husk (1:1) and river sand + coconut husk (1:1). The experiment was in a 2×6 factorial arrangement fitted into a completely randomized design replicated thrice. Results showed that topsoil + coconut husk (1:1) performed best at 4WAP by producing the highest stem height (8.7 cm), stem girth (2.9 cm) number of leaves (3.3), leaf area (41.5 cm^2) and longest root length (14.5) and more roots number (4.1) and this was followed by river sand+coconut husk (1:1) while the sole river sand gave the lowest vegetative traits. There was no significant difference on the vegetative parameters of the two varieties of Heliconia flowers and also on interaction. According to this experiment, topsoil + coconut husk (1:1) growth media is the better one for the growth of these Heliconia two varieties of flowers in the area of study. However, it needs more study on soil to recommend for farmers and the continual use of top soil as a candidate growth medium need to be discouraged because it is non-renewable hence, in the absence of top soil, river sand in combination with coconut husk (1:1) could serve as next available alternative.

Key words: Flowers, Golden Torch, growth, jade, media, responses, varieties.

INTRODUCTION

Flowers are an integral part of human life due to their diversity in beauty, form, texture, color and fragrance (Urooj-Ul-Nissa et al., 2015). Jade (*Heliconia psittacorum*) and Golden Torch (H. *psittacorum* × H. *spathocircinata* are well-known Heliconia cut flowers belonging to the family *Heliconiace*, formaly included in the family Musaceae where they were grouped with the bananas. Heliconia derived from the Greek word *Helikonios,* is a genus of about 100 to 200 species of flowering plants. Heliconia flowers are fairly insignificant. What most people would call the 'flower' is actually a group of colorful specialized leaves, called bracts.

Heliconia leaves look more or less like banana leaves. They are generally green, but some are tinged slightly with colour (particularly when young) and sometimes the leaves and stems are coloured or patterned slightly. The true flowers are hidden inside these bracts. Heliconias are tropical plants related to bananas, cannas and gingers. There are about 100 different individual species, and most species then have a large number of hybrids and cultivars, with flower styles varying significantly from the original. Jade' is a beautiful flower characterized by pale white/cream/yellow bract shading to a light pink towards the edges. It is a vigorous grower and prolific producer of flowers most of the year, and makes an excellent cut flower. It grows about 1 to 2 m high while Golden Torch' grows up to 3 m tall, with bright yellow flowering bracts.

Several authors (Ekwu and Mbah, 2007; Baiyeri and Mbah, 2006) had advocated for the replacement of natural top soil as growth medium of floricultural crops with other available options due to some of its shortcomings such as non-sustainability and as a non-renewable resources. Continuous digging of agricultural soils meant for cropping arable land could make the land susceptible to erosion and other forms of soil degradation. Olosunde et al. (2008) reported other limitations of top soil such as heterogeneity with regard to physical and chemical properties; Water holding capacity and bulk density could hinder the normal growth of floricultural plants. Tariq et al. (2012) reported that although natural top soil is acceptable as a growing medium for plants in garden/field, but it is not the right choice for plants in pots or containers because the frequent water demanded by the container plants will cause soil compaction resulting in a tight and brick-like mass. Since top soils are generally unsatisfactory for the production of plants in containers, because the soils do not provide the aeration, drainage and water holding capacity required for plant growth in order to improve the situation several "soilless" growing media have been developed in which plants are grown. Growing media are materials, other than soils *in situ*, in which plants are grown. There are two types of growth media: organic and inorganic. The organic media used materials like peat, compost, tree bark, coconut (*Cocos nucifera* L.Coir), poultry feathers, wood chip, wood shaving, saw dust, Fleece and Marc. The inorganic media are classified into two namely Natural and Synthetic media. Materials used for inorganic natural medium are Sand, Gravel, Rockwool, Glass wool, Perlite Vermiculite, Pumice, Expanded clay, Zeolite Volcanic tuff and Sepiolite while the synthetic natural media used materials are Foam mats (polyurethane),"Oasis" (plastic foam) Hydrogel (Olympios, 1999). Generally, these media are mixed together rather than used alone, as each usually provides its own function. A perfect growth media performs four (4) functions namely: Serving as a reservoir for plant nutrient, holding of water in a way that makes it available to the plant, provision of gases and water at the same time and act as plant support. Some individual materials (substrates) can provide all four functions, but not at the required level of each. For example, sand provides excellent support and gas exchange but has insufficient water and nutrient supplying capacity. The coarse particles of sand have small surface area per unit of volume compared to the finer parties of soil or peat moss. Since water is held on the surfaces of particles, sand has a small water reserve. Since most nutrients in the sand medium are held in the water films, there is every like hood of little nutrient reserve. It is pertinent to note that a single soil less medium alone cannot supply all the nutrients hence the media are mixed together.

Amendment of growth medium with coarse minerals such as river sand to increase air filled pores and drainage was suggested by Dolor et al. (2009). Hartmann et al. (2007) also noted that an ideal potting medium should provide porosity to allow good aeration. One of the most important criteria for successful germination is a reliable germination medium. The influence of the medium is felt even before the plant sprout, because of its water retention and aeration properties. Locally and readily available materials such as wood shaven, sawdust, rice hull, river sand, coconut fiber and mixture of these materials have been proven to be a good media for germination of many crops (Ekwu and Mbah, 2007). Adams et al. (2003) and Akanbi et al. (2002) cited by Baiyeri (2005) noted that the use of organic material offers a great advantage over the conventional topsoil because it provides adequate nutrients to seedlings, better root substrate relation than conventional soil mix and less pre-dispose the seedlings soil borne pests and diseases. The two varieties of Heliconia flowers were chosen for the study based on their popularity, availability, ornamental value, and commercial profitability of long shelf life, the chosen cultivars are characterized by flowering precocity, relatively compact size, uniformity and diversity of flower color, and abundance of flowers. In addition to the afore mentioned characters, the colours and shapes of Heliconia made popular cut flowers can be used for decoration at occasions and homes. These attractive flowers are relatively cheap and represent good value for money. However, there is dearth of information on the actual growth medium for its propagation. Hence, the objective of this study was to compared the performance of selected growth media on two varieties of Heliconia flowers namely Jade and Golden Torch in south south agro- ecological zones of Nigeria.

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MATERIALS AND METHODS

Experimental site

The experiment was carried out in the Department of Crop and Soil Science Demonstration Plot, Faculty of Agriculture, University of Port Harcourt, Nigeria between 1st of April to 8th of May, 2013. University of Port Harcourt is located at latitude 4° 3" N to 5° N and longitude 6°, 45'E to 70°E, with an average temperature of 27°C, relative humidity of 78% and average annual rainfall between March and November that ranges from 2500 to 4000 mm.

Source of materials

The rhizomes set planted were obtained from within the University of Port Harcourt premises. Each rhizomes set had three nodes and some developed roots at an average weight of 9 kg and average length of 8 cm. The black polythene bags (25 cm × 15 cm × 7cm) used for the experiment were purchased from Choba market in along East West road in Port Harcourt. The topsoil used was collected with soil auger at depth of 0-15 cm from land in the Department of Crop and Soil Science Demonstration Plot, Faculty of Agriculture, University of Port Harcourt. The river sand was obtained from Choba river along East West Road, University of Port Harcourt while the matured brown coconut husk were obtained from a coconut's seller as waste for free of any cost at Ogoni Village in Port Harcourt.

Experimental design, treatment and cultural details

The experimental plot was cleared and mapped out. Clearing was done on the 1st of April 2013. The experiment comprised of two (2) Heliconia varieties (Jade and Golden torch) and six (6) potting media which are itemized as follows:

1. Topsoil (control)
2. River sand
3. Coconut husk
4. Topsoil + River sand (1:1)
5. Topsoil + Coconut husk (1:1)
6. River sand + Coconut husk (1:1

The experiment was in a 2×6 factorial arrangement fitted into a completely randomized design giving a total of twelve (12) treatment combinations replicated thrice. A 2×6×3=36 polythene pots were used for the experiment. Each poly bag presents one treatment which was randomly assigned within each replication.

The potting media were analyzed before sowing for their physical and chemical characteristics using standard Laboratory described by Mylavarapus and Kennelley (2002). The mixed media was measured in equal ratio of 1:1 before it has been put inside the polythene bags. The experimental plot was cleared and mapped out. Clearing was done on the 1st of April 2013. Planting of the H*eliconia psittacorum* rhizomes were done immediately after the poly bags were ready and filled with each substrate on the 10th of April 2013.The polybags were then arranged on thick polythene sheet to prevent the growth of the roots into the ground, to avoid possible uptake of nutrients under partially shaded Oil palm tree of 50% light intensity and spaced at 30 cm × 30 cm. Weeding was done through hand picking; the 1st weeding was done on the 24th of April, 2013; two weeks after planting and the second was on the 8th of May, 2013. The volume of water required to water the growth sources to field capacity was calculated according to Udo et al. (2009). The result obtained from the calculation was 200 ml and it was used to water one (1) polybag in each of the media routinely at alternate day before the termination of the experiment.

Data collected

The data collected were: number of leaves, leaf area, stem height, root length, number of root and stem girth. The three (3) plants which served as replicate per medium were tagged for growth measurement. All the data were collected at weekly interval except number and length of roots. The number of leaves was determined by counting. The leaf area was determined by multiplying leaf length by leaf width and a constant (0.75) (Remison and Lucas, 1982). Stem height was measured from the base of the plant to the tip of the terminal leaf bud with meter rule.

Stem diameter was measured five centimeters above ground level using micrometer screw gauge and converted to girth using the following formula:

Stem girth = Stem diameter × 22/7(π)(Ede et al., 2015)

Number and length of roots was determined at termination of the experiment at four weeks after planting (4WAP) by destructive sample and thereafter root were counted and measured with a meter rule.

Statistical analysis

Data collected were analyzed using the analysis of variance procedure while the means were separated by the least significant differences (LSD) at 5% level of probability.

RESULTS

Physiochemical properties of the selected growth media

Some physiochemical properties of the selected growth media are presented in Table 1. Results showed that the sand particles ranged from 0.00 to 99.1% with river sand producing the highest sand value and the lowest insole coconut medium. Silt particle ranged from 0.00 to 1.8% with top soil producing the highest value and the lowest in sole coconut husk medium. Clay particle followed the same trend as silt particle. The pH value ranged from 5.40 to 7.09 with sole river sand having the highest value and sole top soil the lowest. Total organic carbon ranged from 0.63 to 45.83% with sole coconut medium producing the highest value and sole river sand the lowest. Total nitrogen ranged from 0.09 to 0.52% with sole coconut husk having the highest value and sole river sand the lowest. Available phosphorus (P) followed similar trend with that of total nitrogen Exchangeable Calcium (Ca) ranged from 0.5 to 1.40 cmol/kg with sole coconut husk producing the highest value and sole river sand the lowest. Exchangeable Magnesium (Mg) followed similar trend. Exchangeable Potassium (K) ranged from 0.23 to 3.22 cmol/kg with sole coconut husk producing the highest value and top soil medium the lowest. Electrical conductivity (Ec) ranged from 0.06 to 2.01 dS/m with coconut husk having the highest value and top soil the lowest.

Table 1. Some physico-chemical properties of the selected growth media.

Growth media	Sand (%)	Silt (%)	Clay (%)	pH (H_2O)	TOC (%)	Total N (%)	Avail P (mg/kg)	Exch.Ca (cmol/kg)	Exch.Mg (cmol/kg)	Exch.K (cmol/kg)	EC (dS/m)
Top soil (control)	91.40	1.80	6.80	5.40	3.40	0.13	8.67	0.86	2.02	0.23	0.06
River sand	99.10	0.10	0.80	7.09	0.63	0.09	1.23	0.13	0.18	1.86	0.23
husk	0.00	0.00	0.00	6.80	45.83	0.52	784.50	1.40	2.75	3.22	2.01
Top+River sand (1:1)	95.25	0.95	3.80	6.25	2.02	0.11	4.95	0.50	1.10	1.05	0.15
Topsoil + coconut husk (1: 1)	45.70	0.90	3.40	6.10	24.62	0.32	396.59	1.13	2.39	1.73	1.04
River sand +coconut husk (1: 1)	49.55	0.05	0.40	6.94	23.23	0.31	392.87	0.78	1.47	2.54	1.12

TOC = Total organic carbon, Avail = Available, Exch = Exchangeable and EC = Electrical conductivity.

Table 2. The effect of selected growth media on mean stem height (cm) of two varieties of Heliconia.

Factor	1WAP	2WAP	3WAP	4WAP
Growth media				
Top soil (control)	2.7	2.9	5.5	7.1
River sand	2.0	2.8	5.1	6.9
Coconut husk	2.9	4.1	5.8	7.6
Top soil + River sand (1:1)	3.1	4.7	6.0	8.0
Top soil + coconut husk(1:1)	3.9	6.0	6.9	8.7
River sand+ coconut husk(1:1)	3.5	5.4	6.7	8.3
LSD P = (0.05)	0.84	0.93	0.97	0.29
Variety				
Jade	2.9	4.0	5.7	7.5
Golden Torch	3.0	4.5	6.7	8.0
LSD P = (0.05	NS	NS	NS	NS

[1]Week(s) after planting=WAP; [2]Not significant=NS.

Response of the selected growth media

Stem height and stem girth

The effect of the selected growth media on stem height of the two varieties of Heliconia flowers is presented in Table 2.There were significant ($p< 0.05$) differences in mean stem height among the selected growth media throughout the periods of observation. At 1WAP (Week after planting) mixed top soil and coconut husk (1:1) produced the tallest stem (3.9 cm) which was not statistically different ($p> 0.05$) from river sand+ coconut husk (1:1) (3.5 cm) and Top soil + River sand (1:1) (3.1 cm) while sole river sand had the shortest stem

Table 3. The effect of selected growth media on mean stem girth (cm) of two varieties of Heliconia.

Factor	WAP[1]	2WAP	3WAP	4WAP
Growth media				
Top soil (control)	1.2	1.6	1.9	2.3
River sand	1.1	1.4	1.8	2.2
Coconut husk	1.7	1.9	2.2	2.5
Top soil + River sand (1:1)	1.6	2.0	2.4	2.7
Top soil + coconut husk (1:1)	1.8	2.2	2.5	2.9
River sand+ coconut husk (1:1)	1.8	2.2	2.5	2.8
LSD P = 0.05	0.39	0.30	0.19	0.29
Variety				
Jade	1.6	1.9	2.1	2.3
Golden Torch	1.6	1.8	2.0	2.4
LSD P =0.05	NS	NS	NS	NS

[1]Week(s) after planting, [2]Not significant.

(2.0 cm) which was statistically ($p>0.05$) similar with that of top soil (2.7 cm).

At 2WAP, Top soil + coconut husk (1:1) produced significantly ($p<0.05$) tallest plant (6.0cm) which was statistically at *par* with that of River sand + coconut husk (1:1) (5.4 cm) while sole river sand (2.8 cm) had the shortest plant which was at *par* with top soil (2.9 cm).

At 3WAP, the combination of top soil and coconut husk (1:1) produced significantly tallest plant (6.9 cm) which was statistically similar with mixed river sand+coconut husk (6.7 cm) and Top soil + River sand (1:1) (6.1 cm) while river sand produced the shortest plant (5.1 cm) which was at *par* with those of topsoil (5.5 cm) and sole coconut husk (5.8 cm). At 4WAP (termination period) Top soil + coconut husk(1:1) produced significantly tallest plant of 8.7cm and differed from other media while sole river sand had the shortest plant of 6.9 cm was statistically similar to that of the sole top soil (control) (7.1 cm)

The effect of the selected growth media on stem girth of the two varieties of Heliconia flowers is presented in Table 3. At 1WAP, top soil+coconut husk (1:1) and river sand+coconut husk (1:1) produced significantly highest stem girth of 1.8 cm each which were statistically at par with those of sole coconut husk (1.7 cm) and top soil +river sand (1:1)(1.6 cm) while the shortest stem girth of 1.2 cm and 1.3 cm recorded in sole river sand and top soil respectively. At 2WAP Sole River sand and top soil produced the lowest stem girth of 1.4 and 1.6 cm, respectively and differed significantly from the other medias. At 3WAP, top soil + coconut husk (1:1) and river sand+coconut husk (1:1) had highest stem girth of 2.5 cm but statistically similar with that of top soil + river sand (1:1) (2.4.cm) and differed significantly from the other media. At 4 WAP, sole river sand had the lowest stem girth of 2.2 cm which was statistically similar with that of the sole top soil (control) (2.3 cm) which differed significantly

Number of leaves and leaf area

The effect of selected growth media on number of leaves of two varieties of Heliconia flower is presented in Table 4. There were no significant differences among the growth media in terms of number of leaves throughout the observation periods except at 4WAP, where top soil + coconut husk (1:1) had the highest number of leaves (3.3) but statistically at par with those of river sand+ coconut husk (1:1)(3.2) and Top soil + River sand (1:1) (3.0); while the lowest number of leaves were recorded in sole river sand medium (2.1) which were statistically similar with those of sole top soil (2.2) and coconut husk (2.5).

The effect of the selected growth media on leaf area of the two varieties of Heliconia flower is presented in Table 5. Top soil + coconut husk (1:1) produced significantly greatest leaf area throughout the observation periods while the lowest was in sole river sand.

Root length and number of roots

The effect of selected growth media on root length of the two varieties of Heliconia flower is presented in Table 6. Top soil+coconut husk (1:1)and river sand + coconut husk had the longest plant root of 14.5 and 14.4 cm, respectively and were statistically comparable with that of Top soil and river sand (12.6 cm) medium. Sole river sand produced shortest root length of 10.4 cm which was statistically at par with those of top soil 10.7 cm and sole

Table 4. The effect of selected growth media on mean number of leaves of two varieties of Heliconia flower.

Factor	WAP[1]	2WAP	3WAP	4WAP
Growth media				
Top soil (control)	1.3	1.4	2.0	2.2
River sand	1.2	1.5	1.8	2.1
Coconut husk	1.1	1.5	2.0	2.5
Top soil + River sand (1:1)	1.1	1.6	1.7	3.0
Top soil + coconut husk (1:1)	1.5	1.8	2.0	3.3
River sand+ coconut husk (1:1)	1.2	1.8	1.8	3.2
LSD P = 0.05	NS	NS	NS	0.42
Variety				
Jade	1.0	1.4		2.5
Golden Torch	1..3	1.6		2.5
LSD P =0.05	NS[2]	NS	NS	NS

[1]Week(s) after planting, [2]Not significant.

Table 5. The effect of selected growth media on mean leaf area ($cm^{2)}$ of two varieties of Heliconia flower.

Factor	1WAP[1]	2WAP	3WAP	4WAP
Growth media				
Top soil (control)	4.7	8.5	12.3	19.9
River sand	4.6	7.9	11.2	18.1
Coconut husk	5.1	10.5	16.2	22.0
Top soil + River sand (1:1)	5.3	16.6	19.7	24.2
Top soil + coconut husk (1:1)	16.5	29.8	31.6	41.5
River sand+ coconut husk (1:1)	10.1	20.1	22.4	31.4
LSD P = 0.05	5.60	8.84	8.48	9.96
Variety				
Jade	7.6	14.9	18.7	26.9
Golden Torch	7.9	16.1	19.2	27.4
LSD P =0.05	NS[2]	NS	NS	NS

[1]Week(s) after planting; [2]Not significant.

coconut husk medium (11.1 cm). The effect of the selected growth media on number of root of the two varieties of Heliconia flower showed that there was no significance ($p>0.05$) among the selected growth media though top soil had the highest numbers of root (4.1).

Effect of variety

Vegetative parameters (stem height, stem girth, number of leaves, leaf area, root length and number of roots) as influenced by variety are presented in Tables 2 to 6. There were no significance differences among the vegetative parameters throughout the periods of observation.

Interaction of variety and growth media

Variety x grow media interaction on stem height, stem girth, number of leaves leaf area, number of roots and root length were not significantly ($P < 0.05$) differed throughout the observation periods.

DISCUSSION

Use of suitable growing media or substrates is an essential for production of quality horticultural crop Bhardwaj (2014). Physiochemical properties of the growing media pose their effect on the plant growth (Wilkerson, 2002); therefore the composition of the

Table 6. Effect of selected growth media on mean root length and numbers of two varieties of Heliconia flower.

Factor	Root length (cm)	Number of roots
Growth media		
Top soil (control)	10.7	3.3
River sand	10.4	3.3
Coconut husk	11.1	3.4
Top soil + River sand (1:1)	12.6	3.7
Top soil + coconut husk (1:1)	14.5	4.1
River sand + coconut husk (1:1)	14.4	4.0
LSD P = 0.05	3.16	NS
Variety		
Jade	9.5	3.6
Golden Torch	10..1	3.5
LSD P =0.05	NS	NS[1]

[1]Not significant.

growth media is very important factor to be taken under consideration (Ingram et al., 2003). The selected growth media differed in physical and chemical characteristics. The differences were more pronounced in the chemical characteristics. Olosunde et al. (2015) reported that an effective growth media should provide anchorage to the plant; hold sufficient available nutrients; be porous and well drained; relatively low in soluble salts; standardized and uniform; free pests and pathogens; biologically and chemically stable. The three main components that can bring about to a media's chemical make-up are PH, electrical conductivity (EC) and exchange capacity (CEC). These three components satisfied the acceptable range required for plant growth in a medium as reported by Abad et al. (2002).

The vigorous and fast growth of seedlings in terms of stem height recorded in Top soil + coconut husk(1:1), River sand + coconut husk(1:1) Top soil + River sand (1:1) and Coconut husk may be attributed to better water holding capacity and availability of the nutrients for plant growth. While poor growth in sole river sand and top soil and may be due to low nutritional status for plant offered by the medium. Similar results were reported by Conover and Poole (1981) as better plant height in mixed media than in sole media. The greater stem girth noticed in mixed top soil + coconut husk (1:1), river sand + coconut husk (1:1), top soil + River sand (1:1) and sole coconut husk might be attributed to its richer nutritional status which enhanced photosynthetic activity resulted in more plant stored material, thereby increasing seedling girth. Similarly lesser stem girth in top soil (control) and sole river sand might be due to less soil aeration and poor root penetration which had restricted the plant growth.

Number of leaves was affected by the growth media throughout the period of observations except at 3WAP. The non-significance difference noticed at 3WAP among the media might be attributed to nutrient imbalance. Numbers of leaves or vegetative growth generally depend upon the nutrients taken or absorbed from media in which it's sown. The first stage of growth of the plant usually takes nutrients stored in the rhizomes, when the nutrients are depleted the plant depends upon the root up- take from the soil or from the media. Remison (1997) noted that plants propagated by rhizome have enough storage material that can easily root and forms new shoot. In the same vein, Diaz-Zorita et al. (2005), and Cernac et al. (2006) also stated that germination and seedling emergence is independent of soil nutrient status, but rather depends totally on cotyledons still attached to the seedling which are rich in stored food reserves until the seedling becomes autotrophic and have ability to utilize the food reserves.

The highest number of leaves recorded in Top soil + coconut husk (1:1), river sand+ coconut husk (1:1), top soil+river sand (1:1) and sole coconut husks could be attributed to presence of adequate nutrients. While the lowest value recorded in sole river sand and top soil could be attributed to compactness nature of the soil which restrict nutrient up take. More number of leaves in plants implies good vigor and environmental suitability in growth media. Plant leaves are also important during the process of photosynthesis. More leaves tend to increase photosynthetic activity in plants. The greatest leaf area recorded in mixed Top soil + coconut husk (1:1) media might be due to immediate availability of nutrients and water for plant uptake. The result from the findings are also in support from previous work done by Wuryaningsih et al. (1999) who noted significant increase in pot Anthurium leaf number while using coconut husk as growing media. River sand + coconut husk (1:1) also had

larger leaf area due to its ability to retain nutrient and water for translocation to the shoot system. The number of roots were not affected by the different growth media though Top soil + coconut husk (1:1) and River sand+ coconut husk (1:1) tend to have more number of roots than the other media. This result also in line with that of Waziri et al. (2015) who noted that there were no significant differences in growth media consisting of three different soil types (river sand, top soil and a mixture of river sand + top soil + cow dung in ratio 1:1:1) number of roots of *Delonixregia* stem cuttings. The probable reason for this could be improve in structure and texture of the growth media. Mixed media of Top soil + coconut husk (1:1), River sand+ coconut husk (1:1), Top soil + River sand (1:1) and sole coconut medium had longest root. The reason could be that there were macro pores spaces within the germination media that allows roots growth, since there was no compaction of the media that can restrict the growth of roots as does in sole river sand medium. The slight increase in length observed in sole river sand than sole top soil medium could be attributed to the better aeration and good drainage of water, which could promote root growth and development. Air spaces are required for supply of sufficient oxygen for respiration.

There was no significant difference in the vegetative growth of the two varieties (jade and golden torch) throughout the observation periods probably because both varieties are of same species. Variety x growing media interaction on stem height, stem girth, number of leaves leaf area, number of roots and root length were not significantly ($P < 0.05$) differed throughout the growing periods. This indicated that variety and growth media are independent in their effects on these parameters.

SUMMARY AND CONCLUSION

Outstanding findings drawn from this study showed that the two varieties of heliconia flowers responded well to the selected growth media. Topsoil + Coconut husk (1:1) and River sand + Coconut husk (1:1) were the best candidate media for producing the two flowers judging from their satisfactory growth performances in terms of stem height, stem girth, number of leaves, leaf area, root length and roots number; while top soil and river sand were adjudged as the worst candidate media because of their unsatisfactory growth performance. In conclusion, Topsoil + Coconut husk (1:1) combination is the best growing medium of these two varieties of heliconia in the area of study. However, the continual use of top soil as a candidate growth medium need to be discouraged because of soil degradation, non-sustainability and non-renewability associated with it; hence, in the absence of top soil, river sand in combination with coconut husk (1:1) could serve as next available alternative. Replicate studies on growth response of Heliconia flower varieties to some growth media under field conditions is still needed to provide sufficient information to the farmers in the of study area and other regions with similar geographical axis.

Conflict of Interests

The authors have not declared any conflict of interests.

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