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Research Article
Phylogenetic Relationship and Genetic Variation among *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* Ecotypes in Southwest Nigeria

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Abstract

Background and Objective: *Thaumatococcus daniellii* (*T. daniellii*) and *Megaphrynium macrostachyum* (*M. macrostachyum*) are perennial, understorey herbs with similar morphological and vegetative properties. Genetic relatedness and variation among and within ecotypes of the plant species in Nigeria were studied using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. **Materials and Methods:** Plant samples were collected at random from 24 sites across 5 states in Southwest Nigeria. The DNA was extracted from the leaves by cetyl trimethyl ammonium bromide (CTAB) method and amplified with respective PCR-based primers. Genetic variation within and between the populations was determined by analysis of molecular variance (AMOVA), cluster analysis was performed using unweighted pair group method using arithmetic averages (UPGMA). **Results:** The RAPD and ISSR markers generated clear reproducible bands, polymorphic bands (PPD) produced by RAPD and ISSR markers were 86.7 and 91.3%, respectively. Genetic variation among the two plant populations was 93.68 and 77.87%, respectively with RAPD and ISSR markers, variation within each plant population was 6.32 and 22.13%, respectively for the RAPD and ISSR primers. The cluster analysis indicated that genetic differentiation occurred more among the population than within population of *T. daniellii* and *M. macrostachyum*. **Conclusion:** This study showed that *T. daniellii* and *M. macrostachyum* species are genetically distinct from each other. However, the genetic variation within each of the two species is low, thus, management and conservative approaches are required to preserve and develop the plants for future commercial exploitation.

Key words: *Thaumatococcus daniellii*, *Megaphrynium macrostachyum*, genetic variation, genetic relatedness, RAPD, ISSR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Thaumatococcus and *Megaphrynium* are two important African genera of *Marantaceae*, a family of perennial, understorey herbs from the *Zingiberales* order of flowering plants[1-4]. *Thaumatococcus* was thought to be monotypic, having a single species, *Thaumatococcus daniellii,* until recently when a second species, *Thaumatococcus flavus*, was discovered in Gabon[5]. *Magaphrynium* genus has five species, *Magaphrynium macrostachyum*, *Magaphrynium trichogynum*, *Magaphrynium gabonense*, *Magaphrynium distans* and *Magaphrynium velutinum*[1].

Thaumatococcus daniellii (Benn.) Benth and *Megaphrynium macrostachyum* (K.Schum. Milne-Redh) are widely distributed in rainforest and coastal areas of West and Central Africa. The economic, nutritional and medicinal potentials of these non-timber forest products are yet to be fully exploited[6]. Locals in Southern part of Nigeria where the plants thrive, use their leaves to wrap foods and stalks to thatch house. The potential uses of the plants as a non-wood fiber source and in animal feed are currently being explored[7-9]. *Thaumatococcus daniellii* is the natural source of thaumatin, a globally traded protein sweetener and taste modifier that has now turned the plant into an introduced species in different parts of the world[10]. Thaumatin is harvested from the pyramidal or trigonal-shaped fruits of *T. danielli*.

Thaumatococcus and *Megaphrynium* have similar morphological and vegetative properties. One striking vegetative similarity is the structural nectary located at the tips of cataphylls in these two genera that has not been recorded for other members of the *Marantaceae* family[2]. *Thaumatococcus daniellii* leaf (ewe eran-Yoruba) and *M. macrostachyum* leaf (ewe gbodogi-Yoruba) are similar, except for the larger size and dark band on either side of the midrib of *M. macrostachyum* leaf[11]. In spite of attempts at morphological characterization of *T. daniellii* and *M. macrostachyum* species, knowledge gap exists regarding the genetic relatedness and diversity among and within the two related plant species in Nigeria. The importance of molecular identification and characterization of various plants and animal resources, at the present time, cannot be over emphasized. Most characterization procedures used in time past were based on cytological, morphological and biochemical parameters which are often affected by environmental and several other factors[12].

Developments in molecular biology techniques have improved characterization efficiency, thereby allowing accurate identification of species, ensuring cultivar development and offering certification and protection of breeder's right[13]. A phylogenetic tree, based on DNA sequence data (rps16 intron), was used to resolve major clades in the *Marantaceae* family[1]. In another phylogenetic relationship and classification study, an unexpected sister relationship was found between *Thaumatococcus* and *Megaphrynium* which were classified in the Sarcophrynum clade[3].

A combination of the two molecular markers, Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR), was engaged to achieve the study objective. Random Amplified Polymorphic DNA (RAPD), which involves a single primer with both forward and reverse priming activity, uses small oligonucleotides of random sequence, usually 8-10 bp long, to amplify complementary strands on template DNA[14]. Inter-Simple Sequence Repeat (ISSR), on the other hand, uses primers that may or may not be radioactively labeled, usually repeating sequence of 2-5 nucleotides[15]. The present study is an attempt at reassessing and reconstructing the phylogenetic relationship between the two plant species. The focus was to establish the genetic relatedness and diversity among and within the populations of *T. daniellii* and *M. macrostachyum* found in Southwest Nigeria.

MATERIALS AND METHODS

Chemicals and reagents: Tris-HCl, ethylenediaminetetraacetic acid (EDTA), cetyl trimethyl ammonium bromide (CTAB) and agarose were obtained from Sigma-Aldrich, Germany. The RAPD and ISSR PCR primers were obtained from (Operon Technologies, CA and USA) and Integrated DNA Technology, Iowa, US, respectively, PCR reagents containing *Taq* polymerase, deoxynucleotide triphosphates and PCR buffer where obtained from TransGen Biotech, Inc. Beijing, China, Qiagen (Venlo, The Netherlands). All other chemicals and reagents were of analytical grade.

Collection of plant species: *Thaumatococcus danielli* and *M. macrostachyum* plant samples were collected, from 24 different locations across five states (Ekiti, Ogun, Ondo, Osun and Oyo) in Southwest Nigeria (Fig. 1). The samples were collected from October, 2013 to February, 2015. Collection sites included farms, forest reserves, local markets, open range and botanical gardens of Universities and Research Institutes. The sites were significantly distant, at least 15 km from each other. Random sampling procedure was employed on fields having large population of the plants. Accession numbers such as oyNO001 (Oyo State) and ekNO010 (Ekiti State) were assigned at the point of collection (Table 1). Whole plants were collected from the sites and re-planted in Covenant

Fig. 1: Study area: Ekiti, Ogun, Ondo, Osun and Oyo states in Southwest Nigeria

Table 1: Lists of collected samples showing their accession numbers and their collection sites

Accession code	State	Collection area	Latitude	Longitude	Altitude
TdOy001	Oyo	FRIN	N/A	N/A	N/A
TdOy002	Oyo	Awe Oye	N/A	N/A	N/A
TdOy003	Oyo	Akufo	N/A	N/A	N/A
TdOy004	Oyo	Ogunmakin	N/A	N/A	N/A
TdOn005	Ondo	Onisere	N 06°45.016'	E005°07.090'	72 m
TdOn006	Ondo	Idiroko	N/A	N/A	N/A
TdOn007	Ondo	Okitipupa	N 06°28.972'/	E 004°47.111'	42 m
TdOn008	Ondo	Agoladan	N 06°55.135'	E 004°57.559'	60 m
TdOn009	Ondo	Onisere	N 06°46.172'	E 005°07.188'	82 m
TdOg010	Ogun	Ota	N/A	N/A	N/A
TdOg011	Ogun	Ifo	N/A	N/A	N/A
TdOg012	Ogun	Ode Remo	N/A	N/A	N/A
TdOs013	Osun	Ile-Ife	N/A	N/A	N/A
TdOs014	Osun	Olode	N/A	N/A	N/A
TdOs015	Osun	Moro	N 07°31.752'	E 4°29.849'	48 m
TdOs016	Ekiti	OyeEkiti	N 07°27.768'	E 005°42.916'	549 m
TdOs017	Ekiti	Ayegbaju	N/A	N/A	N/A
TdOs018	Ekiti	Ifaki	N/A	N/A	N/A
TdOs019	Ekiti	Ilupeju	N/A	N/A	N/A
TdOs020	Ekiti	OyeEkiti	N 07°57.421'	E 005°19.626'	448 m
TdOs021	Ekiti	Ishare	N 08°43.025'	E 004°59.365'	202 m
MmOy022	Oyo	FRIN	N/A	N/A	N/A
MmOy023	Oyo	AWE OYE	N/A	N/A	N/A
MmOn024	Ondo	Idiroko	N/A	N/A	N/A
MmOn025	Ondo	Agoladan	N 06°55.135'	E 004°57.559'	60 m
MmOn026	Ondo	Onisere	N 06°46.172'	E 005°07.188'	82 m
MmOg027	Ogun	Ifo	N/A	N/A	N/A
MmOg028	Ogun	Ode Remo	N/A	N/A	N/A
MmOs029	Osun	Ile Ife	N/A	N/A	N/A
MmOs030	Osun	Olode	N/A	N/A	N/A
MmOs031	Osun	Ile Ife	N/A	N/A	N/A
MmOs032	Osun	Ipetumodu	N 07°31.685'	E 004°25.820'	228 m
MmOs033	Osun	Moro	N 07°31.752'	E 4°29.849'	48 m
MmEk034	Ekiti	Ayegbaju	N/A	N/A	N/A
MmEk035	Ekiti	OyeEkiti	N 07°27.768'	E 005°42.916'	549 m
MmEk036	Ekiti	OyeEkiti	N 07°27.768'	E 005°42.916'	549 m

N/A: Not available

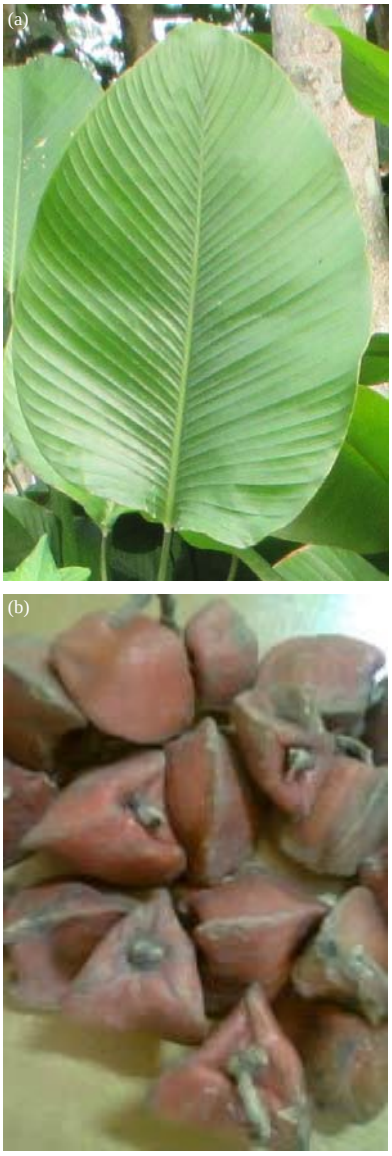

Fig. 2(a-b): *Thaumatococcus daniellii* (Benn.) Benth, (a) Leaf and (b) Fruits

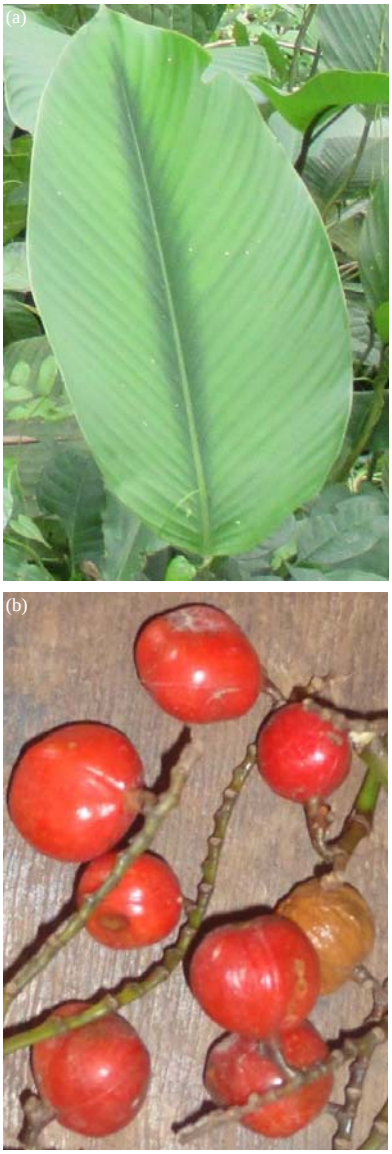

Fig. 3(a-b): *Megaphrynium macrostachyum* (K. Schum.) Milne-Redh, (a) Leaf and (b) Fruits

University garden. The plant species were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria (Fig. 2, 3). The study was carried out at the molecular biology research laboratory of Covenant University from October, 2013 to June, 2015.

DNA extraction: Plant DNA was extracted by a modification of cetyl trimethyl ammonium bromide (CTAB) method of Doyle[16]. Fresh leaves of the plant (0.15 g) were pulverized and homogenized in 1000 µL of CTAB isolation buffer [2% CTAB, 1 M Tris-HCl, pH 8.0, 0.5 M EDTA, pH 8.0, 5 M NaCl and 1% polyvinylpyrrolidone (PVP)]. The mixture was incubated for 15 min at 55°C and centrifuged at 12000 rpm for 5 min. Supernatant was collected and mixed with chloroform: Isoamyl alcohol (24:1 ratio) and centrifuged at 13000 rpm for 1 min. The upper aqueous phase, containing the DNA, was collected. This was mixed with 7.5 M ammonium acetate (950 µL) and absolute ethanol (500 µL) and placed at -20°C for 1 h to precipitate the DNA. The precipitated DNA was isolated by centrifugation at 13000 rpm for 1 min. The DNA pellet was washed twice with 70% ethanol, centrifuged at 13000 rpm for 1 min and dried. This was re-suspended in 50 µL of sterile, DNase-free water containing 10 µL of RNase.

Table 2: Sequence of the selected RAPD and ISSR primers

Marker type	Primer (ID)	Primer sequence (5'-3')	Temperature (°C)
RAPD	OPA-10	GTGATCGCAG	27
	OPB-15	GGAGGGTGTT	27
	OPC-11	AAAGCTGCGG	27
	OPC-20	ACTTCGCCAC	29
ISSR	ISSR 1	$(GA)_6CC$	37
	ISSR 2	$(CAC)_3GC$	40
	ISSR 3	$(CTC)_3GC$	37

RAPD-PCR: The RAPD primers were obtained from Operon Technologies Inc. The sequences of the selected RAPD primers are shown in Table 2. PCR protocol was based on the original method described by Williams *et al.*[17]. Optimal conditions for DNA amplification were empirically determined by testing the primers and different concentrations of genomic DNA (10-50 ng). Amplification reaction mixture (12.5 µL) contained 20 ng DNA, 15 mM $MgCl_2$, 1×PCR buffer [Tris Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, pH 8.7 (Transgene)], 0.2 µM primer, 0.2 mM dNTPs, 1.25 unit *Taq* DNA polymerase and DNAse-free water (to make upto 12.5 µL). DNA amplification was performed in thermal cycler 2000(Bio-Rad thermal cycler), programmed for 1 min initial denaturation/activation at 94°C, 2 min extension at 72°C, 45 cycles of 30 sec at 94°C, 55 sec at annealing temperature and a final extension for 15 min at 72°C. A negative control, which contained all PCR components and water in place of DNA, was used to check DNA contamination of reagents. Amplification products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide and photographed under ultraviolet light. Molecular weights were estimated using a 1 kb DNA ladder.

ISSR- PCR: The ISSR primers were obtained from integrated DNA technology. The sequences of selected ISSR primers are shown in Table 2. The ISSR-PCR was performed using the method of Zietkiewicz *et al.*[18] with modifications. The amplification reaction mixture (25 µL) contained 20 ng DNA, 15 mM $MgCl_2$, 1×PCR buffer [Tris Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, pH 8.7 (Qiagen)], 0.2 µM primer, 0.2 mM dNTPs, 1.25 unit *Taq* DNA polymerase and DNAse-free water (to make upto 25 µL). The amplifications were carried out using a thermal cycler 2000 (Bio-Rad thermal cycler), with an initial denaturation/activation for 5min at 95°C, 1 min extension at 72°C, 40 cycles of 1 min at 94°C, 55 sec at annealing temperature and a final extension for 10 min at 72°C. A negative control was used to check DNA contamination of the reagents. Amplification products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide and photographed under ultraviolet light. Molecular weights were estimated using a 1 kb DNA ladder.

Statistical analysis: Molecular weights of the bands were estimated based on standard bands from 1 kb DNA marker (RAPD: Thermo Scientific, ISSR: TransGen). Clear bands were scored as present (1), absent (0) or missing (?) at particular position migrated on the gel with the use of PyElph Package Software Version 1.4 and visual aid. The data matrix generated was entered into the fingerprint analysis with missing data 1.31 and the software was used to access genetic diversity among and between population samples based on percentage of polymorphic bands (PPB) and analysis of molecular variance (AMOVA) among and within population samples ($p<0.001$).

Genetic similarity matrix was also determined using Fingerprint Analysis with missing Data Software version 1.31[19] based on the Jaccard similarity coefficient[20]. The distance transformation (distance = 1 for similarity) was applied to the similarity matrix and used in cluster analysis to determine similarities between the studied species. The cluster analysis was used to divide data set into subsets while placing the closest accessions together. Collected data were grouped using the unweighted pair group method with arithmetic mean (UPGMA) method of clustering. The UPGMA tree was visualized with TreeView 1.6.6 software and the results were presented as dendrogram[21].

RESULTS

The study area and collection sites in Southwest Nigeria are as shown in Table 1. Figure 1 and 2 shows the pictures of the leaf and fruits of *Thaumatococcus daniellii* (Benn.) Benth. and *Megaphrynium macrostachyum* (K.Schum.) Milne-Redh, respectively.

Primer selection: The primers were screened under optimized conditions, a total of 4 RAPD and 3 ISSR primers were selected based on their ability to generate strong amplification and reproducible products from the plants' DNA. The nucleotide sequence and other properties of the selected RAPD and ISSR primers are shown in Table 2.

Amplified products: Figure 3-5 showed the photographs of amplified products obtained with RAPD primers (OP-B15 and OP-C 20) and ISSR primer (ISSR-3). The molecular weights of the products ranged between 500 and 5000 bp. Numbers 1-36 on the gel represent the numbers serial number of the plant materials collected within Southwest Nigeria.

Table 3 showed the polymorphic and monomorphic bands generated among *T. daniellii* and *M. macrostachyum* samples using selected RAPD and ISSR primers. The RAPD

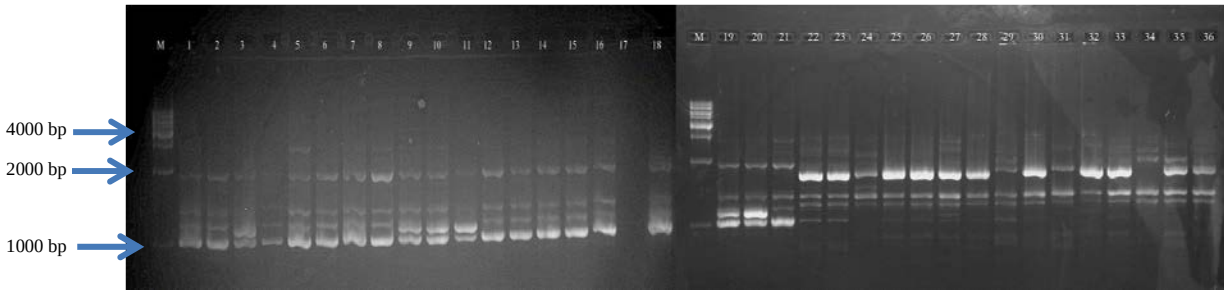

Fig. 4: Amplified products using OP-B15 RAPD primer with molecular weight between 500-4000 bp

Serial number 1-36 on the gel represent samples with the following ascension numbers, TdOy001, TdOy002, Td, Oy003, Td Oy004, TdOn005, TdOn006, TdOn007, TdOn008, TdOn009 TdOg010, TdOg011, TdOg012, TdOs013, TdOs014, TdOs015 TdOs016, TdOs017, TdOs018, TdOs019, TdOs020, TdOs021, MmOy022, MmOy023, MmOn024, MmOn025, MmOn026, MmOg027, MmOg028, MmOs029, MmOs030, MmOs031, MmOs032 MmOs033, MmEk034, MmEk035 and MmEk036, respectively with IKb DNA ladder (TransGen Biotech, Inc.)

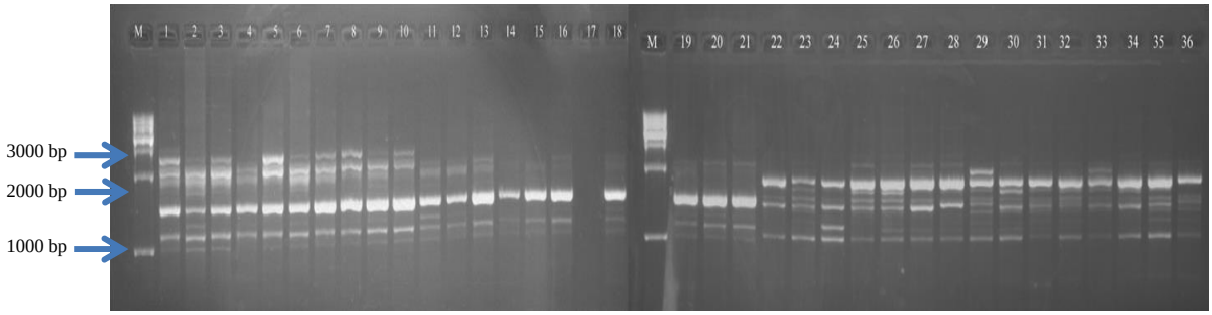

Fig. 5: Amplified products using OP-C 20 RAPD primer with molecular weight between 1000-3000 bp

Serial number 1-36 on the gel represent samples with the following ascension numbers, TdOy001, TdOy002, Td, Oy003, Td Oy004, TdOn005, TdOn006, TdOn007, TdOn008, TdOn009 TdOg010, TdOg011, TdOg012, TdOs013, TdOs014, TdOs015 TdOs016, TdOs017, TdOs018, TdOs019, TdOs020, TdOs021, MmOy022, MmOy023, MmOn024, MmOn025, MmOn026, MmOg027, MmOg028, MmOs029, MmOs030, MmOs031, MmOs032 MmOs033, MmEk034, MmEk035 and MmEk036, respectively with IKb DNA ladder (TransGen Biotech, Inc.)

Table 3: Polymorphic and monomorphic bands generated among *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* samples using selected RAPD and ISSR primers

		Number of bands			
Marker type	Primer (ID)	NPB	NMB	Total	PPB (%)
RAPD	OPA-10	11	1	12	91.7
	OPB-15	11	2	13	84.6
	OPC-11	9	1	10	90.0
	OPC-20	8	2	10	80.0
	Total (4 primers)	39	6	45	86.7
ISSR	ISSR 1	10	4	14	71.4
	ISSR 2	19	-	19	100.0
	ISSR 3	13	-	13	100.0
	Total (3 primers)	42	4	46	91.3

NPB: Number of polymorphic bands, NMB: Number of monomorphic bands, PPB: Percentage of polymorphic bands

primers generated a total of 45 bands, an average of 11.3 bands/primer, among the population samples of the two plant species. Molecular weight of the bands ranged from 500-5000 bp. The RAPD primers produced significant number of polymorphic bands (86.7%) among the two plant species. The ISSR primers produced a total of 46 clear bands, an average of 15.3/primer, with varying molecular weight in the range of 1000-5000. The ISSR primers yielded 91.3% polymorphic bands across the two plant populations.

The bands (polymorphic and monomorphic) generated within populations of *T. daniellii* and *M. macrostachyum* samples using the RAPD and ISSR primers are shown in Table 4. The RAPD gave 42.9 and 38.7% polymorphic bands, respectively for *T. danielli* and *M. macrostachyum* within the population samples of each plant species. The ISSR produced 40.9 and 41.9% polymorphic bands, respectively for *T. danielli* and *M. macrostachyum* within population samples of each plant species. Polymorphic bands within population samples of each plant species were 42.9 and 38.7%, respectively for *T. danielli* and *M. macrostachyum*.

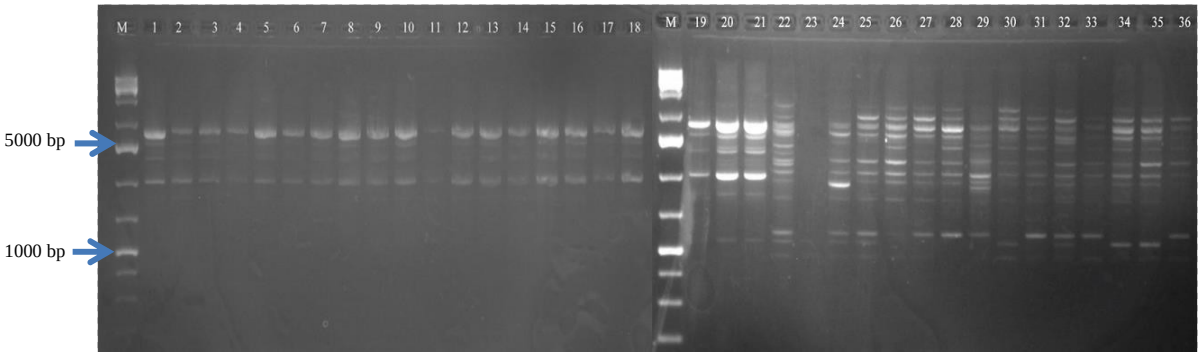

Fig. 6: Amplified products using ISSR-2 primers with molecular weight between 1000-5000 bp

Serial number 1-36 on the gel represent samples with the following ascension numbers, TdOy001, TdOy002, Td, Oy003, Td Oy004, TdOn005, TdOn006, TdOn007, TdOn008, TdOn009 TdOg010, TdOg011, TdOg012, TdOs013, TdOs014, TdOs015 TdOs016, TdOs017, TdOs018, TdOs019, TdOs020, TdOs021, MmOy022, MmOy023, MmOn024, MmOn025, MmOn026, MmOg027, MmOg028, MmOs029, MmOs030, MmOs031, MmOs032 MmOs033, MmEk034, MmEk035 and MmEk036, respectively with IKb DNA ladder (Thermo Scientific)

Table 4: Polymorphic and monomorphic bands generated within populations of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* samples using RAPD and ISSR primers

		Plant species							
		T. daniellii				*M. macrostachyum*			
Marker type	Primer (IDT)	NPB	NMB	Total	PPB (%)	NPB	NMB	Total	PPB (%)
RAPD	OPA-10	4	2	6	66.7	5	4	9	55.6
	OPB-15	2	4	6	33.3	3	7	10	30.0
	OPC-11	3	5	8	37.5	1	5	6	16.7
	OPC-20	3	5	8	37.5	3	3	6	50.0
	Total	12	16	28	42.9	12	19	31	38.7
ISSR	ISSR 1	2	7	9	22.2	3	7	10	30.0
	ISSR 2	4	3	7	57.1	11	3	14	78.6
	ISSR 3	3	3	6	50.0	4	3	7	42.9
	Total	9	13	22	40.9	18	13	31	41.9

NPB: Number of polymorphic bands, NMB: Number of monomorphic bands, PPB: Percentage of polymorphic bands

Table 5: Analysis of molecular variance among and within population samples of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* samples based on ISSR and RAPD profiles

Marker type	Source of variation	Degree of freedom	Sum of square	Variance component	Variation (%)	Phi ST (Φ_{ST}) value
ISSR	Among species	9	5.4074	0.1567	77.87	0.7787
	Within species	26	1.1579	0.0445	22.13	
	Total	35	6.5653	0.2012	100.00	
RAPD	Among species	9	4.8598	0.1493	93.68	0.9367
	Within species	36	0.2619	0.0101	6.32	
	Total	35	5.1217	0.1594	100.00	

Analysis of genetic diversity/similarity: Table 5 showed the analysis of molecular variance (AMOVA) among and within population samples of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* samples based on ISSR and RAPD profiles. The AMOVA based on RAPD data showed a variation of 93.68 and 6.32%, respectively among the two plant populations and within each population. Analysis of molecular variance (AMOVA) based on ISSR data also showed a high percentage of the total genetic diversity among populations of both plant species with a value of 77.87%. The genetic diversity within species was low (22.13%) indicating great similarities within *T. daniellii* and *M. macrostachyum* species. The coefficient of genetic differentiation (Φ_{ST}) among populations was 0.9367 and 0.7787, respectively for RAPD and ISSR.

Dendrogram generated by UPGMA cluster analysis based on the RAPD and ISSR bands are shown in Fig. 6 and 7a,b, respectively. Both dendrograms grouped the sample genotypes into 2 main clusters representing the two plant species, *Thaumatococcus daniellii* and *Megaphrynium macrostachyum*. There are sub-clusters within the two main clusters.

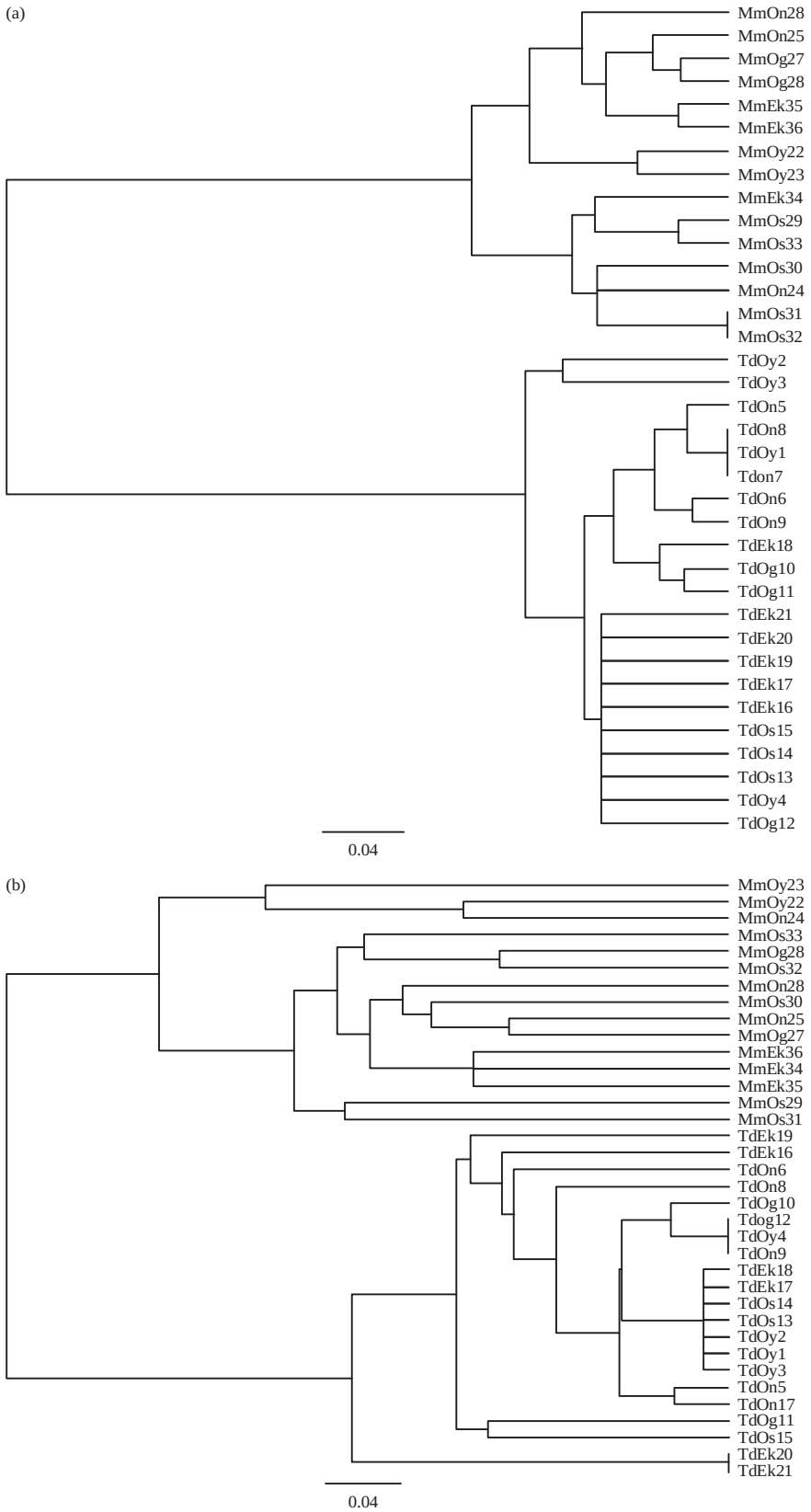

Fig. 7(a-b): Dendrogram generated by UPGMA cluster analysis, based upon (a) 45 RAPD bands and (b) 46 ISSR bands depicting the genetic similarity between the individual plants

DISCUSSION

The study area covered five states in the Yoruba speaking part of Nigeria. The leaves of the two plant species, *T. danielli* (ewe eran) and *M. macrostachyum* (ewe gbodogi) are commercially exploited by the local farmers and traders. Though some local users could identify the two leaf types, many do not consider them to be different as both leaves look much alike and practically serve the same purpose, mainly for wrapping of foods and other items. In spite of the close morphological similarity of the leaves, their fruits are very distinct (Fig. 1, 2). The mature ripe fruits of *T. daniellii* are pyramidal or trigonal in shape whereas *M. macrostachyum* fruits have rounded shape. Moreover, unlike *T. daniellii* fruits that contain thaumatin, *M. macrostachyum* do not produce any known sweetener or taste modifier, this apparently limits its potential for commercial development.

The RAPD and ISSR, since their discovery, have proved to be useful dominant DNA-PCR based markers. They have been used for diverse research purposes, particularly in population genetics as well as in genotoxic and cancer research [22,23]. In the present study, RAPD and ISSR markers have been used to differentiate between two closely similar plant species, *T. daniellii* and *M. macrostachyum.* The study showed that there is a very high genetic variation among the *T. daniellii* and *M. macrostachyum* populations than within the population of each plant species. The selected RAPD primers generated 45 reproducible bands with 39 (86.7%) polymorphic bands. The high percentage polymorphic bands generated by these primers are line with some other diversity study that was based on RAPD marker [24]. The ISSR primers also generated and amplified 46 reproducible bands with 42 (91.3%) polymorphic bands. Rout *et al.*[25] in a study of the genetic relationship among 13 cultivars of *Calathea*, a member of the *Marantaceae* family of plant species, observed 96.1% polymorphism among species studied. The percentage polymorphic bands generated within populations of *T. daniellii* and *M. macrostachyum* was calculated to be 42.99 and 38.7%, respectively with RAPD primers and 40.9 and 64.3%, respectively with ISSR primers. The result showed that the polymorphic loci of the bands generated by RAPD primers were relatively low. A similar result, showing the same dominant properties was obtained among populations of *T. danielliii* using AFLP based markers[26]. Low genetic variation has negative consequences among plant species which include increased mortality rate, slower growth, development instability and greater susceptibility to disease[27-29]. This calls for conservative measures and concerted efforts towards the plants' development to preserve them from extinction.

Furthermore, the RAPD markers revealed a high level variation (93.68%) among species and low level (6.32%) within species. The ISSR markers also showed a high level variation (77.87%) among species and lower level (22.13%) within species. In both markers, the cluster analysis separated the plant species into two major clusters. Standard similarity matrix based on Jaccard's similarity coefficient[20] was generated from the data matrix that was created based on the scored bands. The coefficient of genetic differentiation (Φ_{ST}) among populations was 0.7787. This supports the AMOVA analysis indicating significant genetic diversity among populations with limited diversity within population. All the population samples of *T. daniellii* were in one cluster, the same applies to population samples of *M. macrostachyum*. In each of the clusters, most individuals from the same population formed a group or sub-cluster. The groupings into sub-clusters suggest that the ecotypes could represent the subspecies within the two plant species studied. The similarity of the results from RAPD and ISSR markers indicate that both markers were effective in characterizing the studied species and also authenticate the validity of the analysis.

CONCLUSION

This study showed that *T. daniellii* and *M. macrostachyum* species are genetically distinct from each other. However, the genetic variation within each of the two species is low, thus, management and conservative approaches are required to preserve and develop the plants for future commercial exploitation.

SIGNIFICANCE STATEMENTS

The molecular techniques applied in this study were able to establish the genetic relationship and diversity among and within *T. daniellii* and *M. macrostachyum* ecotypes in Southwest Nigeria. Management and conservative approaches are required to preserve and develop the plants for future commercial exploitation because genetic variation is low within each of the two species.

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