PCR-Based Characterization of Dasheen (*Colocasia* sp.) and Cocoyam (*Xanthosoma* sp.)

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Dasheen (*Colocasia* sp.) and cocoyam (*Xanthosoma* sp.) are two important tuber crops used extensively; they have nutritional and medicinal properties and are used as a source of carbohydrates and calories. Traditional classifications of dasheen and cocoyam are based on morphological, biochemical and agronomical characterization. However, the high degree of morphological and agronomical similarity makes it difficult to distinguish between the two aroids at the species and variety levels. In this study, Random Amplified Polymorphic DNA (RAPD) analyses, UPGMA clustering, -$(CA)_8$RY- microsatellite repeat unit, chloroplast- (*trn*R/Q 01; *trn*L 03/04) and mitochondrial-specific (*NAD* 4.2/4.3; *rps*14/*COB*) primer pairs identified two species of dasheen, one of which has 3 varieties. Of the 18 accessions assessed, 11 were identified as either *C. esculenta* var. *esculenta* or *C. esculenta* var. *antiquorum*. The study also revealed 4 species of *Xanthosoma* including *X violaceae* among the accessions of cocoyam assessed. Random 10-mer primers, -$(CA)_8$RY- repeat, chloroplast- (*trn*R/Q 01) and mitochondrial-specific (*NAD* 4.2/4.3) primer pairs revealed polymorphism in the plastid and mitochondrial genomes that can be used to differentiate between cocoyam and dasheen. The *trn*L 03/04 chloroplast-specific amplification profile of dasheen accession DSC4 identified 2 *trn*L sequences; however, most plants possess a single *trn*L sequence.

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INTRODUCTION

Dasheen (*Colocasia* sp.) and cocoyam (*Xanthosoma* sp.) grow readily in tropical and sub-tropical regions. Dasheen contains large quantities of starch with very small granules making it ideal for use in infant formula and cocoyam is extensively used in therapeutic medicine [1]. In regions where these aroids are widely used traditionally classifications are based on morphological and agronomical characterization such as color, size, shape, texture, position of the leaves, stalk color; corm size and maturity rates. These descriptors are dependent on environmental factors such as rainfall, soil type, shade, elevation, temperature and growing conditions, wetland or dryland. However, morphology changes over the life span of the plant making their use in cultivar identification questionable [2-3]. Biochemical markers are a result of gene activity and are likely to be influenced or regulated by environmental factors which can be difficult to reproduce [4-5]. The use of molecular markers which are unaffected by environmental influences provides an ideal method for more accurately determining the genomic relationship between accessions at the species and subspecies levels [6]. The simplicity and the speed that PCR-based markers discriminated between species make them ideal for handling large numbers of samples. The polymorphisms

revealed by these markers are readily scorable, reducing the effort required for screening. These attributes make them suitable for analyzing genetic diversity [7-10]. Additionally, microsatellite repeats can produce extensive polymorphisms due to site-specific variation as a consequence of the presence of amplifiable repeat units [11-13]. This fact can be exploited to use microsatellites as a differentiator between aroids; the abundance and relative ease of information production by microsatellite markers makes them an ideal tool for plant genetic linkages, physical mapping and cultivar identification. Generally, the majority of microsatellite bands represent a unique locus with two alleles and the absence of a band represents recessive alleles. The present study was designed to exploit the use of microsatellite repeats and PCR-based markers to assess the genetic diversity and relatedness between dasheen and cocoyam.

MATERIALS & METHODS

Sample Collection

Eighteen accessions and dasheen including *C*. *esculenta* var. *esculenta* or *C*. *esculenta* var. *antiquorum*, and 17 cocoyam accessions were collected in Jamaica; *X*. *violaceae*, *Alocasia odorata* and *A*. *cons* were obtained from Wye College, University of London, freeze dried and stored at -20°C (Table 1).

Plant DNA Isolation

DNA was isolated from dasheen and cocoyam accessions using a modified CTAB-based procedure [14]. Briefly, 3g of ground leaves in 30ml of extraction buffer (containing 3% CTAB, 1.4M NACL, 20mM EDTA, 100mM Tris-HCl, 1.0% PVP-40T and 0.2% 2-mercaptoethanol) was incubated at room temperature (RT) and added to wet chloroform:octanol. The aqueous phase was separated by centrifugation and washed with chloroform before incubating at RT in cold iso-propanol. The DNA was collected by centrifugation and dissolved in TE.

Mini preparation: DNA was extracted from 120–150mg dasheen and cocoyam leaf using a mini-scale version of the CTAB-based method.

Spun Column Chromatography DNA purification

DNA was purified by Spun Column Chromatography, a technique developed in the Molecular Diagnostic Laboratory at Wye College, University of London. Briefly, a 0.5ml eppendorf tube with a pin-hole and 10µl of 80-100µm glass bead was placed in a bottomless 1.5ml eppendorf tube. Sepharose CL– 6B/TE slurry was slowly added to the beads and the mixture centrifuged at 2,000rpm. The purified DNA was collected by centrifugation using a Sepharose CL–6B mesh.

PCR-based DNA fingerprinting analyses

10-mer primers: A modified William *et al.* protocol [15] was used for DNA amplification. The amplification mixture contained 10X PCR buffer, 50mM $MgCl_2$, 10mM dNTP, 10µg/µl primer (Table 2), 5U *Taq* polymerase, and 20ng template DNA in sterile de-ionized water. The reaction mixture was overlaid with mineral oil and amplified using initial denaturation at 95°C for 6 min; 45 cycles of 30 sec denaturation at 95°C, 30 sec annealing at 35°C, and 60 sec extension at 72°C; and final extension at 72°C for 10 min.

3'-anchored microsatellite primers: The –$(CA)_8$RY repeat subunit was used for microsatellite analyses. With the exception of 0.2ng template DNA, the amplification mixture was similar to that used for the 10-mer primers. The PCR condition was an initial denaturation at 95°C for 5 min; 40 cycles of 30 sec denaturation

Table 1. Local names and source of samples studied

Dasheen Samples			Cocoyam Samples		Samples from Wye College	
Accession No.	Accession code	Species	Accession no.	Accession code	Accession code	Local name
1	DW		19	XSC1	AC	*Aloccasia cons*
2	DC1	-	20	XSC2	XV	*X. violaceum*
3	DC2	-	21	XM1	AO	*Alocasia odorata*
4	DSC1	-	22	XC		
5	DSC2	-	23	XM2		
6	DM1	-	24	XM3		
7	DM2	-	25	XM4		
8	DM3	-	26	XM5		
9	DSC3		27	XM6		
10	DSC4	-	28	XM7		
11	DC3	-	29	XM8		
12	DSA	-	30	XM9		
13	DSC5	-	31	XM10		
14	DM4	-	32	XM11		
15	DSV	(*C. esculenta* var. *antiquorum)*	33	XSA1		
16	DC4	-	34	XSA2		
17	DC5	(C. *esculenta* var. *esculenta)*	35	XStA		
18	DSJ	-				

Table 2. Sequences of the Operon F-series 10-mer primers used in RAPD analyses

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
OPF-01	ACGGATCCTG	OPF-11	TTGGTACCCC
OPF-02	GAGGATCCCT	OPF-12	ACGGTACCAG
OPF-03	CCTGATCAGG	OPF-13	GGCTGCAGAA
OPF-04	GGTGATCAGG	OPF-14	TGCTGCAGGT
OPF-05	CCGAATTCCC	OPF-15	CCAGTACTCC
OPF-06	GGGAATTCGG	OPF-16	GGAGTCTGG
OPF-07	CCGATATCCC	OPF-17	AACCCGGGAA
OPF-08	GGGATATCGG	OPF-18	TTCCGGGTT
OPF-09	CCAAGTCTTC	OPF-19	CCTCTAGACC
OPF-10	GGAAGCTTGG	OPF-20	GGTCTAGAGG

at 94°C, 30 sec annealing at 59°C, and 2 min extension at 72°C; and final extension at 72°C for 10 min.

Chloroplast and mitochondrial specific primers: The amplification mixture and conditions were similar to those used for 10-mer primer. The sequences of the chloroplast and mitochondrial specific primers are shown in Table 3.

Table 3. Chloroplast- and mitochondria- specific primers

Primer	Sequence
Chloroplast genome	
trnL-03	CGAAATCGGTAGACGCTACG
trnL-04	GGGATAGAGGGACTTGAAC
trnQ-01	GGGACGGAAGGATTCGAACC
trnR-01	ATTCGTCCAATAGGATTTGAA
Mitochondrial genome	
rps14	CACGGGTCGCCCTCGTTCCG
COB	GTGTGGAGGATATAGGTTGT
NAD 4.2	CTCCTCAGTAGCCCATATGA
NAD 4.3	AACCAGTCCATGATCTAACA

Analyses of PCR product

Amplified DNA data analyses: Positions of openly scorable amplified fragments were converted into binary character matrix where "0" represents the absence and "1" represents the presence of a band. Pairwise distance using simple matching coefficient was obtained with TREECON software and evolutionary trees based on distance data generated by neighbor-joining [16-17].

Restriction digestion of chloroplast and mitochondrial amplified fragment: A modified Sambrook *et al.* procedure [18] was used for restriction digestion. Reaction mixture consisting of 10X enzyme buffer, 15µl amplified chloroplast or mitochondrial fragment, 5U *Alu*I, *EcoR*I or *Pst*I restriction enzyme and de-ionized water was incubated at 37°C for 3 hrs and detected by gel electrophoresis.

RESULTS

DNA isolation

Using the modified CTAB-based protocol for DNA isolation from dasheen, cocoyam and *Alocasia* sp. yielded >3000g DNA from 3.0g of tissue and 20µg from 120mg of tissue for large- and mini-preparation, respectively. The method proved to be efficient, easily adapted to microcentrifuge tubes and facilitated large numbers of samples.

RAPD analysis

Based on prescreening of random primers that produced at least 6 easily scorable fragments, primers OPF02, OPF06 and OPF13 were selected to amplify accessions of DNA from dasheen while primers OPF08, OPF09, OPF10 and OPF13 were used for amplification of cocoyam accessions. For primer OPF06 dasheen accessions DSC1, DSC3 and DW were not amplified, and accessions DSA, DSV, DC4, DC5, DSC4, DSC5, and DM4 produced similar RAPD profiles (Figure 1). Similar observations were made for primers OPF02 and OPF13. Amplification of cocoyam accessions using primers OPF08, OPF09, OPF10 and OPF13 produced 4 distinct banding patterns individually. With the exception of XSC1, XSA1 and XSA2, cocoyam accessions produced similar OPF08 amplification profiles. The 0.6kb OPF08-amplified fragment was less intense in accessions XM1 and XM7-XM9. An additional >1.5kb fragment was present in accessions XM6, XM10 and XM11 and was either less intense or absent in accessions XC and XM2-XM5 (Figure 2A). The OPF09 RAPD profiles for the cocoyam accessions were identical with the exception of the profiles for

accessions XSC1, XSA1 and XSA2 which were distinct (Figure 2B). Similar were made for RAPD profiles produced by primers OPF10 and OPF13.

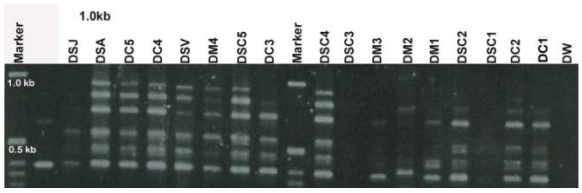

Figure 1. Dasheen DNA Fingerprints: Agarose gel electrophoresis of RAPD polymorphisms obtained with OPF02.

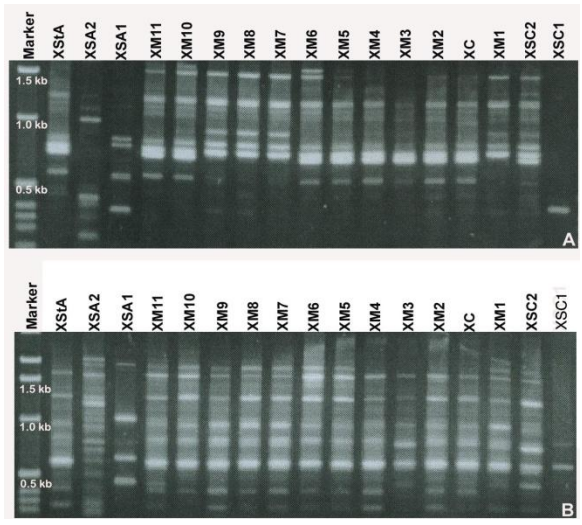

Figure 2. Cocoyam RAPD profiles: Agarose gel electrophoresis of RAPD polymorphisms obtained with primers OPF08 (A) and OPF09 (B); similar profiles were obtained using OPF10 and OPF13. With the exception of accessions XSC1, XSA1 and XSA2 which had distinct amplification profiles, a consistent profile was obtained for the accessions of cocoyams analyzed.

Cluster analysis

Similarity coefficient and genetic variation for OPF02 RAPD pattern ranged from 0-1.0 and 0-100, respectively. A similarity coefficient of 0 and genetic distance of 100 were observed for accessions DW and DSC3 while values of ≤0.4 and ≤60 were obtained for accession DSC1 (Tables 4A & 4B). Similarity coefficient of ≥0.8 and genetic distance of ≤20 were obtained for accessions DC1–DC5, DSC4–DSC5, DSV, DSJ, DSA, DM1 and DM. UPGMA and neighbor-joining cluster analyses indicated close genetic similarities between accessions DC1-DC3, DC5, DSC2, DM4 and DSV (Figure 3). Accessions DC4, DSA, DSC4 and DSC5 were clustered together; there was no genetic distance between accessions DC4 and DSA suggesting a high level of relatedness. Similarly, there was no genetic distance between accessions DCSC3 and DW which were clustered with accessions DSJ, DM1, DM3, and DSC1. Accession DM2 was not clustered with any other accession implying a large genetic distance. Analysis using similarity coefficient indicated that there were 2 groups of dasheen, one of which had 3 subgroups (Table 4 & Figure 3).

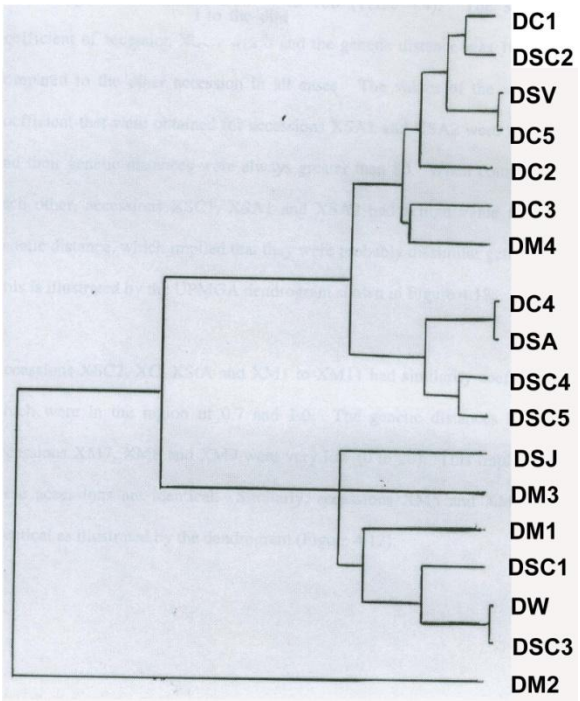

Figure 3. UPMGA cluster analysis fro dasheen: The neighbor-joining analyses revealed close genetic similarities between the dasheen accessions. Accessions DC1-DC3, DC5, DSC2, DM4 and DSV (CEA) were clustered together; accessions DC4, DSA (CEE), DSC4 and DSC5 were clustered together and no genetic distance existed between accessions DC4 and DSA (CEE) indicating a high level of relatedness. Furthermore, there was no genetic distance between accessions DCSC3 and DW which were clustered with accessions DSJ, DM1, DM3, and DSC1. DM2.

Table 4. Similarity matrix data for dasheen

A

	DW	DC1	DC2	DSC1	DSC2	DM1	DM2	DM3	DSC3	DSC4	DC3	DSC5	DM4	DSV	DC4	DC5	DSA	DSJ
DW																		
DC1	0																	
DC2	0	1.0																
DSC1	0	0.33	0.33															
DSC2	0	1.0	1.0	0.33														
DM1	0	0.83	0.83	0.17	0.83													
DM2	0	0.45	0.45	0.09	0.45	0.36												
DM3	0	0.50	0.50	0.25	0.50	0.60	0.18											
DSC3	0	0		0	0	0	0	0										
DSC4	0	1.0	0.45	0.33	1.0	0.83	0.45	0.50	0									
DC3	0	1.0	1.0	0.33	1.0	0.83	0.45	0.50	0	1.0								
DSC5	0	1.0	1.0	0.33	1.0	0.83	0.45	0.50	0	1.0	1.0							
DM4	0	1.0	1.0	0.33	1.0	0.83	0.45	0.50	0	1.0	1.0	1.0						
DSV	0	1.0	1.0	0.33	1.0	0.83	0.45	0.50	0	1.0	1.0	1.0	1.0					
DC4	0	1.0	1.0	0.33	1.0	0.83	0.45	0.50	0	1.0	1.0	1.0	1.0	1.0				
DC5	0	1.0	1.0	0.33	1.0	0.83	0.45	0.50	0	1.0	1.0	1.0	1.0	1.0	1.0			
DSA	0	0.86	0.86	0.29	0.86	0.71	0.55	0.49	0	0.86	0.86	0.86	0.86	0.86	0.86	0.86		
DSJ	0	0.83	0.83	0.40	0.83	1.0	0.33	0.60	0	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	

B

	DW	DC1	DC2	DSC1	DSC2	DM1	DM2	DM3	DSC3	DSC4	DC3	DSC5	DM4	DSV	DC4	DC5	DSA	DSJ
DW	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
DC1		0	0	67	0	17	55	50	100	0	0	0	0	0	0	0	14	17
DC2			0	67	0	17	55	50	100	0	0	0	0	0	0	0	14	17
DSC1				0	67	83	91	75	100	67	67	67	67	67	67	67	71	60
DSC2					0	17	55	50	100	0	0	0	0	0	0	0	14	17
DM1						0	64	40	100	17	17	17	17	17	17	17	29	0
DM2							0	82	100	55	55	55	55	55	55	55	45	64
DM3								0	100	50	50	50	50	50	50	50	51	40
DSC3									0	100	100	100	100	100	100	100	100	100
DSC4										0	0	0	0	0	0	0	14	17
DC3											0	0	0	0	0	0	14	17
DSC5												0	0	0	0	0	14	17
DM4													0	0	0	0	14	17
DSV														0	0	0	14	17
DC4															0	0	14	17
DC5																0	14	17
DSA																	0	14
DSJ																		0

The similarity coefficient value (A) and genetic distance (B) for primer OPF02 amplification of the dasheen accessions ranged from 0 - 1 and 0 – 100, respectively. The lowest similarity coefficient value and consequently lowest genetic distance was obtained for DW, DSC1, DSC3 and DM4. The largest genetic distance was observed for accession DM2.

Cocoyam accession XSC1 had similarity coefficient of 0 and genetic distance of 100 while the similarity coefficient and genetic distance for accessions XSA1 and XSA2 were <0.2 and >80, respectively (Tables 5A & 5B). Accessions XSC2, XC, XStA and XM1-XM11 had similarity coefficients ranging from 0.7-1.0. The genetic distance between accessions XM7-XM9 was 0; similar values were obtained for accessions XM5 and XM6. This suggested that these accessions were identical and was confirmed by cluster analysis. The UPGMA analyses clustered accessions XM5 and XM6, and accessions XM7-XM9 together (Figure 4). While accessions XSA1, XSA2 and XSC1 formed 3 distinct groups, the other cocoyam accessions

Table 5. Similarity matrix data for cocoyam

A

	XSC1	XSC2	XM1	XC	XM2	XM3	XM4	XM5	XM6	XM7	XM8	XM9	XM10	XM11	XSA1	XSA2	XStA
XSC1																	
XSC2	0																
XM1	0	0.80															
XC	0	0.77	0.71														
XM2	0	0.77	0.71	1.0													
XM3	0	0.31	0.29	0.40	0.40												
XM4	0	0.77	0.71	1.0	1.0	0.40											
XM5	0	0.77	0.71	1.0	1.0	0.40	1.0										
XM6	0	0.86	0.93	0.77	0.77	0.31	0.77	0.77									
XM7	0	0.80	1.0	0.72	0.72	0.29	0.72	0.72	0.93								
XM8	0	0.80	1.0	0.72	0.72	0.29	0.72	0.72	0.93	1.0							
XM9	0	0.80	1.0	0.72	0.72	0.29	0.72	0.72	0.93	1.0	1.0						
XM10	0	0.92	0.86	0.83	0.83	0.33	0.83	0.83	0.92	0.86	0.86	0.86					
XM11	0	0.86	0.93	0.77	0.77	0.31	0.77	0.77	1.0	0.93	0.93	0.93	0.92				
XSA1	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0			
XSA2	0	0.20	0.27	0.36	0.36	0.50	0.36	0.36	0.13	0.27	0.27	0.27	0.31	0.13	0.17		
XStA	0	0.06	0.29	0.17	0.17	0.14	0.17	0.17	0.21	0.29	0.29	0.29	0.14	0.21	0	0.29	

B

	XSC1	XSC2	XM1	XC	XM2	XM3	XM4	XM5	XM6	XM7	XM8	XM9	XM10	XM11	XSA1	XSA2	XStA
XSC1	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
XSC2		0	20	23	23	69	23	23	14	20	20	20	8	14	93	80	94
XM1			0	29	29	71	29	29	7	0	0	0	24	7	100	73	71
XC				0	0	60	0	0	23	18	18	18	17	23	100	64	87
XM2					0	60	0	0	23	18	18	18	17	23	100	64	87
XM3						0	60	60	69	71	71	71	67	69	100	50	84
XM4							0	0	23	18	18	18	17	23	100	64	87
XM5								0	23	18	18	18	17	23	100	64	87
XM6									0	7	7	7	8	0	100	87	79
XM7										0	0	0	14	7	100	73	71
XM8											0	0	14	7	100	73	71
XM9												0	14	7	100	73	71
XM10													0	8	100	69	84
XM11														0	100	87	79
XSA1															0	83	100
XSA2																0	71
XStA																	0

The similarity coefficient value (A) and genetic distance (B) for primer OPF08, OPF09, OPF10 and OPF13 amplification of the cocoyam accessions ranged from 0-1 and 0–100, respectively. The lowest similarity coefficient values obtained for XSC1, XSA1 and XSA2 were low and their genetic distances greater than 80. XSC2, XC, XStA and XM1–XM11 had similarity coefficient values between 0.7 and 1.0 (A); the genetic distance between XM7, XM 8 and XM9 was 0–20 and XM5 and XM6 similar genetic distance (B).

clustered together suggesting possible varieties of the same species. OPF08-, OPF09-, OPF010- and OPF13-RAPD profiles for accessions XC, XM3, XM4, XM5 and XStA were similar to X. violaceae indicating the possibility of the same variety (Figure 5). Accessions XCS1, XSA1 and XSA2 produced distinct banding patterns distinct compared to either *X. violaceae* or *Alocasia* sp. Comparison of the chloroplast- and mitochondrial-specific amplification profiles for dasheen and cocoyam to *A*. *cons*, *A*. *odorata* and *X violaceae* indicated that accessions XC, XSC2 and XM1–XM11 produced a 0.6kb fragment similar to *X violaceae* **(**Figures 5B & C).

-(CA)$_8$RY amplification

The -(CA)$_8$RY microsatellite amplified DNA from cocoyam accessions but not dasheen. Cocoyam accessions XSA1, XM3 and XSC2 had distinct profiles, accessions XSC1, XM10, XM11 and XSA1 were not amplified, and the other cocoyam accessions had similar -(CA)$_8$RY profiles (Figure 6). The size of the amplified fragments varied from 0.5- to >1.5kb and included a >0.5-, 1.0- and 1.5-kb fragment in most profiles. The ~1.4kb fragment was produced by accessions XM1 and XM5-XM9, and the 1.0kb fragment was absent from accessions XSC1, XSC2 and XM2.

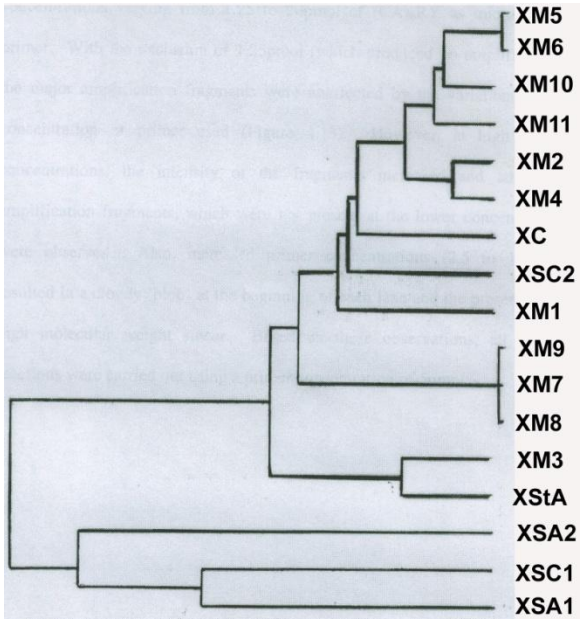

Figure 4. UPMGA cluster analysis fro cocoyam: The neighbor-joining analyses revealed close genetic similarities between the cocoyam accessions; XSC1, XSA1 and XSA2 were distinct from the other cocoyam accessions. Accessions XSC2, XC, XStA and XM1–XM11 were clustered together with accessions XM7, XM 8 and XM9 and XM5 and XM6 being genetically similar.

Genetic diversity in the chloroplast and mitochondria DNA

The mitochondrial-specific primer pair, *NAD* 4.2/4.3, which amplifies intronic sequences between exons 2 and 3 of *NADH-ubiquinone oxidoreductase* 4 gene was unable to amplify either the dasheen or cocoyam accessions (Figure 7A). Chloroplast-specific primer pair *trnQ*/*R* 01 was unable to amplify DNA isolated from dasheen; however, a single 0.5kb fragment was amplified from cocoyam accessions (Figure 7A). The primer pair *trn*L 03/04 amplified a 0.6kb chloroplast fragment from the dasheen accessions and produced a doublet for accession DSC4. The *rps*14*/COB* primer pair amplified a 1.0kb mitochondrial fragment from dasheen; however, the fragment was less intense for DW, DC1 and DC2, and DM1 was not amplified (Figure 7B). With the exception of accessions XSC1, XSA1, and XSA2, primer pair *trn*L 03/04 amplified a 0.6kb chloroplast fragment from cocoyam and primer pair *rps*14*/COB* amplified a 1.0kb mitochondrial fragment.

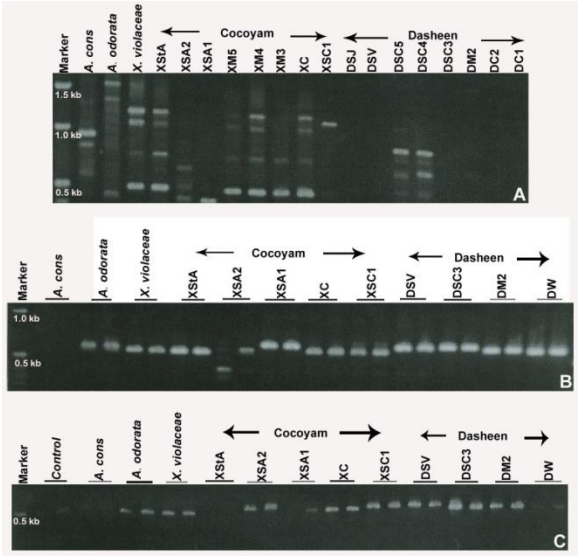

Figure 5. Amplification of *trnL* 03/04- and *rps*14/*COB*-specific fragments: With the exception of accessions XSC1, XSA1 and XSA2 which had distinct profiles, the OPF02 (A), *trnL* 03/04 chloroplast-specific (B) and *rps*14/*COB* mitochondrial-specific (C) amplification profile for accessions of cocoyams analyzed was identical to *X. violaceae* (A).

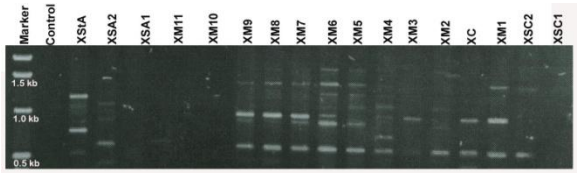

Figure 6. -$(CA)_8$RY microsatellite amplification: Agarose gel electrophoresis of **-(**$CA)_8$RY microsatellite amplification of cocoyam; with the exception accessions XSA1 and XSA2 which had distinct amplification profiles, a consistent profile was obtained for the accessions of cocoyams analyzed. Dasheen accessions were not amplified by **-(**$CA)_8$RY microsatellite repeat.

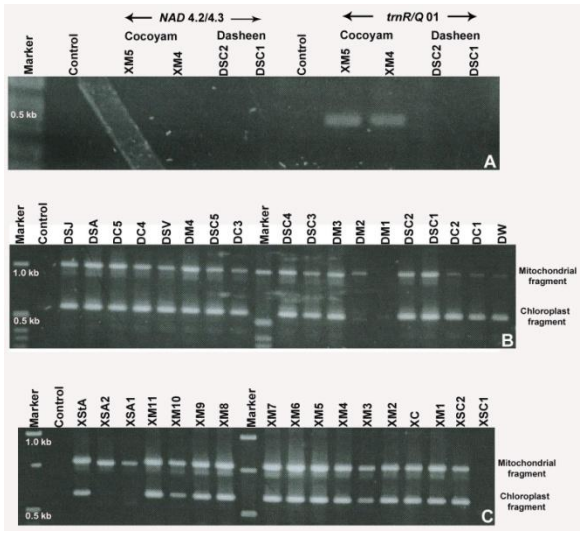

Figure 7. Chloroplast and mitochondrial amplification: Mitochondrial primer pair, *NAD* 4.2/4.3, was unable to amplify either the dasheen or cocoyam accessions. The chloroplast primer pair, *trn*R/Q 01, amplified the cocoyam accessions but not dasheen accessions (A) and can be used to differentiate between the two. Primer pair *rps*14/*COB* (mitochondrial) and *trnL* 03/04 (chloroplast) was unable to amplify accession DM2 (B). Cocoyam accession XSC1 was not amplified by the mitochondrial primer pair, *rps*14/*COB*, and the fragment produced by XSA1 and XSA2 was smaller than the fragment produced by the other accessions. Primer pair *trnL* 03/04 amplification of accessions XSC1, XSA1 & XSA2 produced a 'doublet'(C).

Restriction digestion of chloroplast– and mitochondria–specific fragments

*Pst*I was capable of digesting the *trn*L 03/04 chloroplast fragments and *Eco*RI digested the *rps14*/*COB* mitochondrial fragments; however, both the *trn*L 03/04 chloroplast and *rps14*/*COB* mitochondrial fragments were digested by *Alu*I. *Alu*I restriction of the *rps14/COB* fragment produced 0.2- and 0.7-kb fragments for dasheen and cocoyam. However, the *Alu*I digestion of the *trn*L03/04 chloroplast fragment revealed marked differences: dasheen produced 0.2- and 0.40-kb digested fragments while cocoyam accessions XSC, XSA1 and XSA2 produced an additional 0.5kb fragment. *Eco*RI digestion of the *trn*L fragment produced 0.2- and 0.4-kb fragments for dasheen accessions DSC1, DM2 and DSC4; however, DM2 produced an additional 0.4kb fragment. The amplified dasheen and cocoyam *rps14/COB* mitochondrial fragments did not have a *Pst*I digestion site, but the *trn*L03/04 chloroplast fragments produced a 1.0kb *Pst*I digested fragment for cocoyam.

DISCUSSION

PCR-based techniques including RAPD, -$(CA)_8$RY microsatellite repeat, chloroplast– and mitochondrial–specific primers were used for cultivar identification and differentiation and to assess the genetic relatedness among species of dasheens and cocoyams. Accessions DW and DM3 were not amplified with either OPF02 or OPF06, and the banding pattern obtained by accessions DM4 produced a distinct pattern suggesting differences between the dasheen accessions assessed. Moreover, differences in the OPF06 and OPF13 RAPD profiles produced by accessions DC1-DC4, DC5, DSC2, DSC4, DSC5, DSA, DSV and DM4 indicated that these are possible varieties of the same *Colocasia* sp. The RAPD data was confirmed using similarity matrix. Intra- and inter-specific cultivar polymorphisms were easily distinguished and indicated 2 groups of dasheen, one of which had 3 subgroups. The genetic distance is low and the similarity coefficient is high indicating that with the exception of accession DM2, the dasheen accessions were varieties of *C. esulenta*. Accessions DSV and DSA were known

to be *C. esulenta* var. *antiquorum* and *C. esulenta* var. *esulenta*, respectively. Accession DC5 was clustered with accession DSV and thus was identified as *C. esulenta* var. *antiquorum*. Additionally, other members of the UPGMA cluster, accessions DC1-DC3, DSC2 and DM4, are closely related to *C. esulenta* var. *antiquorum* and represented a subgroup. Accession DC4 was clustered with DSA, *C. esulenta* var. *esulenta*, and along with accessions DSC4 and DSC5 formed another subgroup. Accessions DSJ, DM3, DM1, DSC1, DW and DSC3 formed the final subgroup of *C. esulenta*.

The (CA) motif which belongs to a gene that codes for the small subunit of ribulose triphosphate was unable to amplify dasheen suggesting that (CA) is not the most frequent microsatellite repeat in dasheen and can be used to discriminate between dasheen and cocoyam. Microsatellites can be used as a DNA-marker for germplasm collection; however, to maximize the use of tandem repeats as primers, some awareness of the most frequent repeats in plant species is essential. Additionally, with the exception of accession DM2, similar *rps*14/*COB* mitochondria fragments were amplified from dasheen suggesting possible varieties of *C. esculenta*. Dasheen accessions DM2 produced qualitatively and quantitatively distinct mitochondrial fragment suggesting either distinct species or distant-related varieties of *C. esculenta*. This confirms observations made using RAPD, (CA) repeat and UPGMA cluster analyses that implied that the mitochondrial genomes of the dasheen accessions are similar to *C. esculenta*; since mitochondrial size differences are more pronounced between rather than within species [9-13; 19-20]. RAPD analyses of 17 accessions of cocoyam revealed 4 different species or distantly related varieties of the same species. Comparison of the OPF02, OPF08, OPF09 and OPF10 banding patterns of accessions XStA, XSC2 and XM1-XM11 with *X. violaceae* indicated a high degree of similarity implying different varieties of the same species. However, accessions XSC2, XM1-XM11 and XStA produced similar banding patterns; accessions XM7, XM8 and XM9 produced identical amplification profiles as did accessions XM5 and XM6. The RAPD profiles of accessions XSC1, XSA1 and XSA2 were quantitatively and qualitatively different from the other accessions of cocoyam suggesting different species or distant-related varieties.

The genetic distance between accessions XSC2, XM1-XM11 and XStA was ≤10% suggesting a high degree of similarity. Compared to the other accessions, the genetic distance between accessions XSC1, XSA1 and XSA2 was ≥80% indicating either distinct species or distant-related varieties [16]. These distinctions were further demonstrated by UPMGA cluster analyses which revealed 4 groups; accessions XSC2, XM1-XM11 and XStA produced similar amplifications to and were identified as varieties of *X. violaceae*. The results obtained from RAPD and UPGMA analysis of cocoyam accessions were confirmed by microsatellite (CA) amplification. The CA amplification produced distinct profiles for accessions XSC1, XSA1, XSA2 and the other cocoyam accessions [11-13; 19]. Variations observed in the chloroplast *trn*L 3/4 fragments produced by accessions XSC1, XSA1 and XSA2 supported the hypothesis that they are either distinct species of *Xanthosoma* or distant-related varieties of *X. violaceae*. Cocoyam accessions XM1–XM11, XSC2 and XStA produced *rps*14/*COB* mitochondrial fragment similar to *X. violaceae* thus confirming the RAPD, microsatellite and UPGMA cluster analyzes.

Accessions XSA1 and XSA2 yielded a slightly smaller fragment and XSC1 did not produce a *rps*14/*COB* mitochondria fragment. The differences in the sizes of the fragments are indicative of either species or distant-related varieties. PCR based analyses are not influenced by developmental and environmental changes and are a convenient marker for assessing the genetic diversity of dasheen and cocoyam. 10-mer primers were used to randomly amplify dasheen and cocoyam producing polymorphisms that appeared as quantitative and qualitative differences which can serve as a convenient marker for assessing genetic diversity [9-10; 15; 21]. The sizes of the chloroplast and mitochondria fragments amplified from dasheen and cocoyam genomes suggested taxonomically differences. The chloroplast specific *trn*Q/R 01 primer pair amplified DNA from cocoyam but not from dasheen; therefore, it can be used for differentiation.

The *trn*L 03/04 chloroplast specific amplification of dasheen accession DSC4 produced two fragments. The similarity in the fragment intensities could be a result of amplification of sequences at the same location within the chloroplast as opposed to different parts of the organelle. If one fragment was amplified from sequence within the nuclear or mitochondrial genomes there would be marked differences in the intensities of the two fragments as a result of the ratio of nuclear DNA:chloroplast DNA or mitochondrial DNA:chloroplast DNA. The additional *trn*L fragment could be a result of the fact that plastids rely on the nuclear genome for the determination of some functions and are not autonomous organelles. This gives rise to three possibilities: the size of the plastome is insufficient to code for all the known chloroplast-located nucleic acids and proteins; genes determining plastid development and function are inherited in a Mendelian fashion which indicated that they are in the nucleus; or nuclear-coded proteins are translated outside the chloroplast and are subsequently transported across the chloroplast envelope [22]. The chloroplast genome has two inverted copies repeat (IR) which are separated from each other by a long copy sequence (LSC) on one side and a single copy region sequence (SSC) on the other side [23]. It is possible for sequences within the chloroplast genome to be repeated in either of the inverted repeats; such sequences are recognized by the primer, then both regions of the chloroplast genome will be amplified. The extent of the amplifications will be influenced by the frequency of the sequence within the specified region of the chloroplast and will be visualized by size variation of the amplified fragments. Dasheen and cocoyam are usually vegetatively propagated; however, the rare possibility exists for seeds-grown plants. This can result in genetic diversity between seed-grown and vegetatively-propagated plants [24].

The size of the mitochondrial *rps*14/*COB* amplified fragment is conserved across species of dasheen and cocoyam. The highly conserved mitochondrial fragment across species suggests intron encoded function, protein coding regions and conservation in the length and sequence of the gene. Analyses of dasheen and cocoyam using chloroplast– and mitochondrial–specific primers revealed polymorphism, displayed the presence and/or absence of bands, size differences and/or restriction site polymorphisms. Prior to the introduction of DNA-based markers, proteins and isoenzyme markers formed the basis for auditing the genetic diversity of plant species [25]. However, the number of informative markers did not

provide a direct assessment of the potential variations within species at the genomic level.

CONCLUSION

RAPD, UPGMA clustering, -$(CA)_8$RY-microsatellite, chloroplast- (*trn*R/Q 01 and *trn*L 03/04) and mitochondrial-specific (*NAD* 4.2/4.3 and *rps*14/*COB*) primer pairs identified *C. esculenta* var. *esculenta* and *C. esculenta* var. *antiquorum*, and *X violaceae* as species of dasheen and cocoyam, respectively. Chloroplast- (*trn*R/Q 01) and mitochondrial-specific (*NAD* 4.2/4.3) primer revealed polymorphism within plastid and mitochondrial genomes that can be used to differentiate between the genetic relatedness of dasheen and cocoyam. *NAD* 4.2/4.3 which was unable to amplify neither cocoyam nor dasheen cannot be used to distinguish between these species. *trn*Q/R 01 amplified cocoyam but not dasheen and can be used for differentiation. The *trn*L 03/04 chloroplast amplification of dasheen accession DM4 indicated two *trn*L sequences whereas most plants possess only one.

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