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Molecular Variability of *Ocimum gratissimum* L. Accessions Using RAPD Marker

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the molecular variability among accessions of *Ocimum gratissimum* from selected states in Nigeria and Mali using RAPD marker**.**
Study Design: The experimental design was complete randomized design (CRD) with three replicates.
Materials and Methods: Twenty accessions of *Ocimum gratissimum* were collected from nineteen selected Local Governments in four South-western States of Nigeria (Ogun, Oyo, Osun and Lagos) and Mali, to assess their genetic diversity and phylogenetic relationship. Molecular statistics of binary data generated from Random Amplified Polymorphic DNA (RAPD) marker was conducted using numerical taxonomic and multivariate analysis (NTSYS-PC) package, while dendrogram was constructed by Jaccard's similarity coefficient using unweighted paired group method of arithmetic mean (UPGMA).
Results: Accession Y3 from Ona-Ara yielded the highest total volume of DNA concentration (736.9 µl), while the highest genomic DNA concentration of 2.44 ng/μl was recorded in accession L-04 from Agege.

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Out of total number of 52 bands from three primers of RAPD, 48 produced polymorphic amplified products. OPO-08 primer was highly polymorphic with 94.73%, and had the highest allele numbers, gene diversity and polymorphic information contents of 16.0, 0.914 and 0.909 respectively, while OPO-06 produced the highest number of 20 polymorphic bands. Cluster II was the highest group in the dendogram, and comprised of two states (Oyo and Lagos) and Mali which constituted seven accessions; Y-03 (Ona-Ara), Y-04 (Egbeda), Y-05 (Ido), L-01 (Surulere), L-03 (Ifako-Ijaye), L-04 (Agege) and M (Mali).
Accession S-03 from Ife-North was the most distant with highest similarity index of 1.188.
Conclusion: The RAPD is highly polymorphic, and could be useful in characterizing and revealing wide range of genomic variation and phylogenetic relationship among different accessions of *O. gratissimum* with broad genetic base.

Keywords: Ocimum gratissimum; RAPD primers; polymorphism; phylogenetic; variability.

1. INTRODUCTION

Ocimum gratissimum L. is an herbaceous plant in the family of Lamiaceae commonly called African Basil or Scent Leaf. In South-Western part of Nigeria, the plant is called “effinrin-nla” by the Yoruba speaking tribe. The Igbos called it “Ahuji”, while the Hausa in the Northern part of Nigeria refer to it as “Daidoya” [1]. It is widely naturalized in many regions but indigenous to tropical areas especially India, and also in savanna and coastal areas of Nigeria.

Ocimum gratissimum is a shrubbery, perennial herb and woody at the base with stem 1-3m long. Its leaves are usually broad and narrowly ovate, usually 5-13cm long; 3-9cm wide. Both surfaces of the leaf are copiously granular punctuate, in which the upper surface is globulate to sparsely puberulent, while the lower surface is puberulent on veins with margins serrate, apex acuminate, base cuneate and petiole 1- 6cm long. The calyx of *O. gratissimum* is 3-5cm long, enlarging up toca. 7mm long in fruit, tube usually 2-2.5mm long at anthesis, sparsely hipidulous dotted with sessile oil globules, glabrous within [2]. Most of its species grown by traditional farmers are aromatic, important for human consumption and source of spices, folk medicines and fragrance [3].

The species of *O. gratissimum* are underutilized with under-exploited potential for contributing to food security, poverty alleviation, nutritional, medicinal, income generation and environmental services [4,5]. The utilization of *O. gratissimum* in traditional medicine was found to be effective and cheap in fast developing countries due to poor economic situation, expensive and inadequate availability of drugs [6]. Various ailments treated with *O. gratissimum* include; skin diseases, respiratory infections, diarrhoea, fever, eye infections, wound, external and internal parasites, poor milk secretion, poor weight gain, loss of appetite, fatigue among others [7].

Ocimum gratissimum had been used extensively as traditional medicine, condiment and culinary purpose, while inhabitants use a decoction of *O. gratissimum* roots as a sedative for children [8]. Their flowers and leaves are rich in essential oils used in preparation of teas and infusion [9]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea [1]. In the Savanna areas of Nigeria, decoctions of the leaves are used to treat mental illness [10]. The plant is used by the Igbos of South eastern Nigeria in management of the baby’s cord in order to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh [11]. The leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, and are also used for treatment of abdominal pains, disease of the eyes, skin and ear infections, conjunctivitis, pneumonia, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum [12]. The leaf extract is used in treatment of diarrhoea, while the leaf infusions of *O. gratissimum* are used for the relief of stomach upset and haemorrhoids, as pulmonary antisepticum, antitussivum and antispasmodicum [13-15]. Formulations of the leaf essential oil of *O. gratissimum* have been used as topical antiseptics, and in the treatment of minor wounds, boils and pimples [16].

The assessment of genetic diversity is essential to develop improved crop varieties for enhancememt of molecular breeding germplasm,

conservation and management. The genetic diversity of crops can be determined based on pedigree records, nutritional compositions, phytochemical components, morphological traits and molecular markers [17,18]. Molecular markers are more reliable than other methods. Among several other molecular markers, Polymerase Chain Reaction (PCR) based on Random Amplified Polymorphic DNA (RAPD) had been used to assess the genetic variability in many plants. A major advantage of RAPD over many other DNA-based markers is that they require no prior sequence information and no prior knowledge about any particular gene in the target taxon. Therefore, RAPD markers were considered best to characterize landraces, accessions, wild plants or new plant species genetically [19]. Furthermore, PCR method uses lower amount of genomic DNA, and are non-radioactive, but can be developed rapidly with relatively low cost. The rapid increase in the utilization of genus *O. gratissimum*, which resulted to its being threatened, and limited information on genetic characterization based on RAPD necessitated this study. This study therefore investigated the molecular variability among accessions of *O. gratissimum* from selected states in Nigeria and Mali using RAPD marker.

2. MATERIALS AND METHODS

2.1 Collection, Experimental Location, Seed Processing and Planting Method

The dried and matured seeds of *O. gratissimum* were collected from nineteen different locations in the Southwest parts of Nigeria while one sourced from National Horticultural Research Institute (NIHORT) originated from Mali. The collected seeds were processed by vigorous rubbing between palms to retrieve from shaft, afterwards painstakingly sorted. The seeds were again re-dried and preserved in an envelope at room temperature (25ºC). The seeds were planted out in twenty partitions in the research farm of the Department of Botany, University of Ibadan, located in the rainforest area of Southwestern Nigeria. The young fresh leaves of 2 weeks old for each accession were harvested separately into an ice pack for isolation of genomic DNA at the Bioscience unit of International Institute of Tropical Agriculture (IITA), Ibadan Nigeria.

2.2 Genomic DNA Extraction

Total genomic DNA was extracted using modified extraction protocol [20]. The fresh leaves of each sample were ground in pre-heated mortal by adding little extraction buffer (Sodium Dodecyl Sulfate-SDS).

After thorough grinding 500µlof the extraction buffer was added with 5µl of mercaptoethanol also added making the ground leaves slurry, which was then dispensed into eppendorf tube each for the labeled samples. The eppendorf tubes were incubated in water bath at 65ºC for 25 minutes, and vortexed for 10 mins. 200µl of potassium acetate (KAC) was added to each tube containing 350µl of chloroform isoamyl alcohol (CIA, 24:1 v/v), and well mixed before further incubation on ice for 20 minutes. The samples were centrifuged at 3500rpm for 20 minutes. 200µl of 4% PVP was added to the supernatant carefully transferred to newly labeled eppendorf tubes before adding 350µl of CIA and mixed with vortex.

The supernatant was spun with centrifuge at 3500rpm for 30 minutes. The supernatant was again transferred into newly labeled tubes, and then 400µl of isopropanol was added. The samples were then incubated at -80ºC for 4 hours before centrifuging at 3500rpm for 30 minutes. Supernatant was decanted, and then ethanol wash (70%) 300µl was conducted then spun with centrifuge at full speed. The ethanol was also decanted, leaving pellet in each eppendorf tube. The pellets were air dried. 97µl of water and 3µl of RNase were added and decanted. TBE buffer was added to dissolve the pellets by keeping overnight at 37ºC.

2.3 Determination of Concentration and Purity of DNA Sample Using Nanodrop

UV spectrophotometer was used for this measurement. 2µl of TBE, being the buffer used for the DNA storage, was used to initiate the process. The TBE was used as blank on the lower pedester of the spectrophotometer. After blanking, each DNA samples' concentration and purity was measured by applying 2µl of the sample on the lower pedester. The concentration and purity was displayed on the monitor.

2.3.1 Primers optimisation

Primer optimisation was done with ten (10) arbitrary decamer oligonucleotide primers which

were; OPO 01, OPO 06, OPO 08, OPB 10, OPB 14, OPB 19, OPH 02, OPH 05, OPT 07 and OPT 07. These primers were considered based on the information obtained from polymorphism as reported by Singh et al. [21]. Master-mix was prepared for the primers that gave reproducible and scorable amplification for the accessions, and run on PCR machine to check for primers with complementary base pairs with DNA samples. Agarose gel electrophoresis was conducted on the amplified samples, of which the whole selected primers would be found polymorphic.

2.3.2 RAPD-PCR amplifications

The purpose is to amplify lots of double stranded DNA fragments with same identical size and sequence by enzymatic method and cycling conditions. Separation of the DNA double-stranded template, Primer annealing and Extension of new DNA strand by DNA polymerase and deoxyribonucleoside triphosphate (dNTPs) were carried out using the following chemical components and Enzyme; (Taq polymerase), Buffers and $MgCl_2$ (-100 mM Tris-HCl, pH 8.3, -500mM KCl, -15 mM $MgCl_2$, -other (optional), deoxynucleoside Triphosphates (dNTP), Template DNA and Primers (OPO 01, OPO 06, OPO 08). The amplifications were performed in 25 µl containing 50 to 200 ng DNA, PCR buffer (50 mM $MgCl_2$ 1.2 µl, 10xNH_4 buffer 2.5 µl, dNTP 2.0 µl, DMSO 1.0 µl, Taq DNA polymerase 0.1 µl, Primer 1.5 µl, H_2O 14.74 µl, DNA 2.0 µl. The amplifications were preformed in thermocycler programmed 40 cycles of denaturing for 20 seconds at 94ºC, annealing was done for 40 seconds at 37^{o}C and extension for 1 min at 72ºC.

2.3.3 Agarose gel electrophoresis and RAPD-PCR product resolution

Agarose Gel Electrophoresis was first conducted as a quick check for the presence of DNA in the extracted plant samples. 1g of agarose was dissolved in 100ml of 0.5xTBE buffer in a microwave oven for 5minutes. It was cooled in running tape water for 1 minute. 5µl of ethidium bromide, a staining dye, was added to the agarose gel solution casted into the agarose gel plate, before placing in the electrophoresis tank where 5µl of the dyed extracted DNA was loaded and run for 45minutes. The gel was visualized by UV light and photographed using Gel Documentation System. A negative control lacking template DNA was also used in each set. RAPD-PCR products were resolved by electrophoresis on 1.5 % agarose gels in 1 X TBE (Tris-Borate- Ethylene) buffer by running at 150 V for 3:30 hrs. Products were visualized under UV light after staining for 30 mins in a 1 µg/mL ethidium bromide, and then photographed using a UV light machine Bio Doc-It System UVP printer (Mitsubishi). Products were sized against 1000 bp ladder.

2.3.4 Scoring of bands and statistical analysis of RAPD products

The scoring of total bands was done visually from the primer combination and polymorphic band as discrete variables using 1 for the presence of bands and 0 for the absence of bands. The data generated based on binary similarity matrix were analyzed using Numerical Taxonomic System of Statistics (NTSYS) (version 2.21) and Power Marker Software (version 3.25) [22]. Jaccard coefficient of similarity was used to estimate the genetic distance [23], while construction of dendrogram was done by Unweighted Pair Group Method of Arithmetic Means (UPGMA) to establish the phylogenetic relationship among the accessions.

3. RESULTS AND DISCUSSION

The result in Table 2 shows that Accession Y-03 (Ona-Ara) yielded the highest total volume of DNA concentration of 736.9µ/l, while Y-05 (Ido) had the least (48.3 µg/l) though, low but had good genomic DNA concentration. Accession L-04 (Agege) produced the highest genomic DNA concentration of 2.44ng/µl, while Y-04 (Egbeda) had the least (1.68ng/µl).

Banding patterns of three RAPD primers; OPO-01, OPO-06 and OPO-08 are shown in Plate 1. Sizes of amplified bands were estimated using Gel Pro Analyser as 100bp, 200bp, 300bp, 400bp, 500bp, 650bp, 850bp, 1000bp and 1650bp. Each of the *Ocimum gratissimum* accessions was combined as an individual operational unit (OTU). The amplicons observed ranged from 140-1650bp. The largest amplicon was1650bp, and was amplified by OPO-06 primer, while the shortest amplicon 140bp was also amplified by the OPO-06 primer. All the primers of RAPD marker considered in the study were found to produce bands except accession G-06 (Mile 2, Abeokuta North- Ogun) which showed no band.

Table 1. Locations of *Ocimum gratissimum* seed samples

State	Local govt.	Town	Code	GPS-LAT	GPS-LON
OGUN	Ijebu-Ode	Ijebu Ode	G-01	$6^0$49'19.2832''N	$3^0$54'50.7471''E
	Obafemi-Owode	Adigbe	G-02	$6^0$56'57.0332''N	$3^0$30'20.8701''E
	Odeda	Obantoko	G-03	$7^0$14'0.3613''N	$3^0$31'34.2559''E
	Imeko Afon	Imeko	G-04	$7^0$24'59.9746''N	$2^0$45'0.043''E
	Ifo	Iyana'logbo	G-05	$6^0$48'45.5938''N	$3^0$11'50.9766''E
	Abeokuta North	Mile 2	G-06	$7^0$8'45.0996''N	$3^0$19'.3779''E
OYO	Ogbomosho North	Adeniran	Y-01	$8^0$9'28.8711''N	$4^0$15'55.1807''E
	Ogbomosho South	Osupa	Y-02	$8^0$7'0.1895''N	$4^0$12'59.8525''E
	Ona-Ara	Olunloyo	Y-03	$7^0$20'22.8555''N	$3^0$37'44.5859''E
	Egbeda	Idioro	Y-04	$7^0$22'46.6895''N	$3^0$58'2.9814''N
	Ido	Apete	Y-05	$7^0$26'59.9688''N	$3^0$52'12.9004''E
	Ejigbo	Ejigbo	S-01	$7^0$53'45.7383''N	$4^0$19'0.3672''E
OSUN	Iwo	Iwo	S-02	$7^0$37'51.8672''N	$4^0$1052.9346''E
	Ife North	Edun-Abon	S-03	$7^0$32'25.8711''N	$4^0$27'16.1094''E
	Ilesha-East	Omi-Ashoro	S-04	$7^0$36'7.7734''N	$4^0$43'58.9258''E
LAGOS	Surulere	Surelere	L-01	$6^0$29'23.1699''N	$3^0$20'51.0918''E
	Ikeja	Allen	L-02	$6^0$35'45.707''N	$3^0$21'21.833''E
	Ifako ijaye	U-Turn	L-03	$6^0$41'4.6875''N	$3^0$17'18.403''E
	Agege	Orile	L-04	$6^0$37'43.5547''N	$3^0$18'39.3994''E
MALI	National Horticultural Research Institute (NIHORT)		M	$1^0$832'47''N	$1^0$15'18.450''W

Table 2. Nanospectrophotometeric and concentration of extracted DNA from *Ocimum gratissimum* accessions

Ocimum gratissimum accessions	Total volume of DNA concentration (µ/l)	Genomic DNA concentration 260/280purity (ng/µl)
G-01	425.5	1.97
G-02	123.8	1.73
G-03	210.2	1.83
G-04	176.1	1.94
G-05	131.1	1.96
G-06	336.4	2.07
S-01	239.2	2.09
S-02	197.9	2.1
S-03	668.8	1.98
S-04	326.8	2.1
Y-01	233	2
Y-02	302.2	1.98
Y-03	736.9	2.05
Y-04	111.8	1.68
Y-05	48.3	1.73
L-01	270.6	2.14
L-02	172.3	2.07
L-03	191.6	2.02
L-04	101.7	2.44
M	291.6	2.15

G-01 (Ijebu-Ode); G-02 (Obafemi-Owode); G-03 (Odeda); G-04 (Imeko-Afon); G-05 (Ifo); G-06 (Abeokuta North); Y-01 (Ogbomosho North); Y-02 (Ogbomosho South); Y-03(Ona-Ara); Y4-0 (Egbeda); Y-05 (Ido); S-01 (Ejigbo); S-02 (Iwo); S-03 (Ife-North); S-04 (Ilesha-East); L-01 (Surulere); L-02 (Ikeja); L-03 (Ifako ijaye); L-04 (Agege); M (Mali).

A total of 242 scorable bands were obtained. The mean percentage polymorphic bands were 92.30%. A total number of 52 bands were generated, out of which 48 were polymorphic. OPO-08 produced the highest percentage polymorphism, allele number and gene diversity of 94.73%, 16 and 0.9141 respectively, while OPO-06 and OPO-01 had the same percentage polymorphism, major allele frequency and allele number of 90.90%, 0.2632 and 11.000 respectively.

The mean Polymorphic Information Content (PIC) of 0.86 was generated in which the three primers OPO- 01, OPO-06 and OPO-08 were highly polymorphic. Although, primer OPO-08 had the highest PIC value of 0.9088, followed by OPO-01 with 0.8516, while OPO-06 had the least with PIC of 0.8195. Again, OPO- 06 primer produced the highest number of scorable bands out of which 22 were polymorphic, while the least number of bands and polymorphic bands of 11 and 10 respectively were recorded for OPO-01 primer.

The result in Fig. 1 is a dendrogram showing the relationship among the accessions that was constructed from Jaccard's similarity coefficient using Unweighted Paired Group method of Arithmetic mean (UPGMA). Genetic similarity among the accessions ranged from 0.01 to 0.82.The accessions were delineated into three cluster groups. Cluster-I comprised of three accessions (S-01, G-04 and G-02), Cluster-II constituted seven accessions (M, L-01, L-03, Y-04, L-02, Y-05 and Y-03), while Cluster-III consisted of six accessions (Y-02, S-04, Y-01, S-02, G-03 and G-05). The cluster-II and cluster-III are closely related than cluster-I which is further apart from any of the other two clusters irrespective of their geographical locations. Clustering is not related in any way to geographical distribution because Mali accession belong to the same cluster group with accessions from Oyo (Y-03, Y-04and Y-05) and Lagos (L-01, L-02 and L-03), whereas, accession L-04 (Agege) is distinct on its own. Accessions G-01, L-04 and S-03 did not cluster into any of the three cluster groups, while S-03 (Ife-North) was the most distant among them.

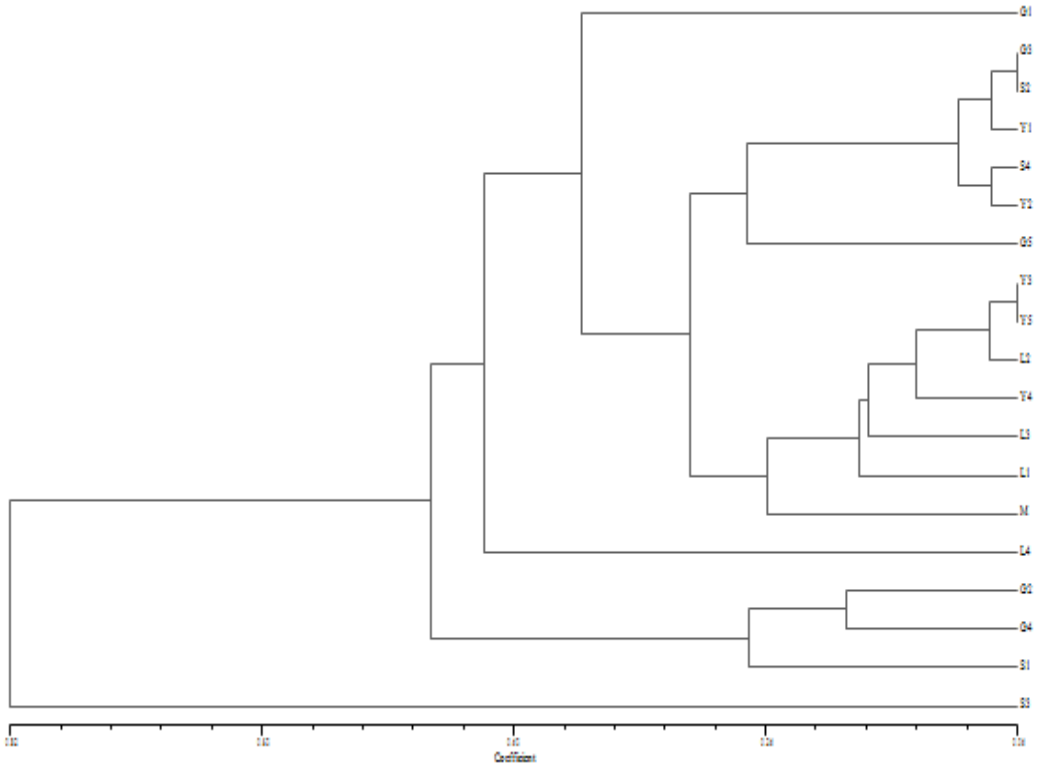

Fig. 1. Jaccard's similarity dendrogram of *Ocimum gratissimum* accessions based on RAPD marker

Table 3. Oligonucleotide sequences, polymorphic information contents, allele number and gene diversity of RAPD primers

RAPD primers	Primer sequence	Major allele frequency	Sample size	Allele number	Gene diversity	Polymorphic Information Content	No of Bands	Number Polymorphic Bands	Percentage Polymorphic Bands (%)
OPO-01	5'GGG CCA CTC A3'	0.2632	19	11	0.8643	0.8516	11	10	90.9
OPO-06	5'CAA GGG CAGA3'	0.2632	19	11	0.8366	0.8195	22	20	90.9
OPO-08	5'CCT CCA GTG T 3'	0.2105	19	16	0.9141	0.9088	19	18	94.73
Total		0.7369	57	38	2.615	2.5799	52	48	92.3
Mean		0.2456	19	12	0.8717	0.86	17.73	16	92.3

Table 4. Genetic distance showing relationship among *Ocimum gratissimum* accessions

	G1	G2	G3	G4	G5	S1	S2	S3	S4	Y1	Y2	Y3	Y4	Y5	L1	L2	L3	L4	M
G1	0																		
G2	0.46																		
G3	0.30	0.30																	
G4	0.72	0.17	0.36																
G5	0.54	0.33	0.25	0.22															
S1	0.82	0.28	0.43	0.22	0.33														
S2	0.25	0.36	0.04	0.43	0.25	0.50													
S3	0.88	0.77	0.82	0.77	0.88	0.88	0.72												
S4	0.28	0.40	0.10	0.46	0.28	0.46	0.06	0.77											
Y1	0.28	0.33	0.06	0.33	0.17	0.40	0.06	0.67	0.13										
Y2	0.20	0.43	0.08	0.50	0.30	0.58	0.04	0.72	0.06	0.10									
Y3	0.40	0.46	0.20	0.46	0.33	0.40	0.20	0.77	0.22	0.13	0.25								
Y4	0.43	0.43	0.28	0.43	0.50	0.43	0.33	0.82	0.36	0.25	0.40	0.10							
Y5	0.40	0.54	0.20	0.54	0.33	0.46	0.20	0.77	0.22	0.13	0.25	0.04	0.10						
L1	0.33	0.82	0.36	0.62	0.54	0.62	0.30	0.77	0.28	0.28	0.25	0.17	0.20	0.13					
L2	0.40	0.54	0.20	0.46	0.33	0.40	0.20	0.77	0.22	0.13	0.25	0.08	0.15	0.04	0.13				
L3	0.67	0.58	0.33	0.43	0.58	0.50	0.40	0.82	0.43	0.30	0.46	0.20	0.13	0.15	0.20	0.15			
L4	0.82	0.82	0.36	0.82	0.72	0.72	0.43	1.19	0.46	0.40	0.50	0.33	0.43	0.33	0.54	0.40	0.36		
M	0.46	0.54	0.25	0.54	0.46	0.54	0.25	1.02	0.28	0.22	0.30	0.13	0.25	0.17	0.33	0.22	0.30	0.28	0.00

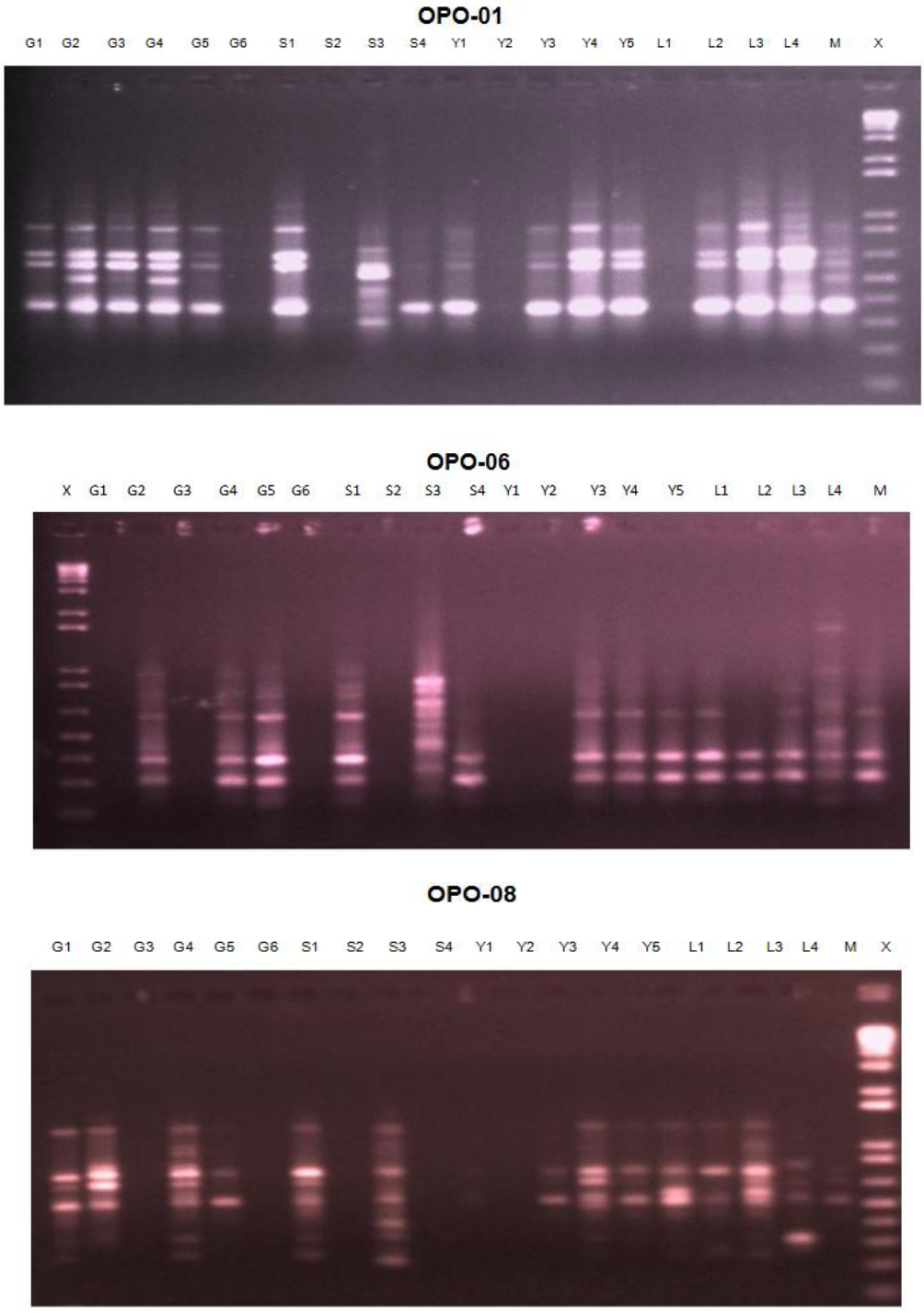

Plate 1. RAPD pattern of *Ocimum gratissimum* on Agarose gel under Gel-doc generated by OPO-01, OPO-06 and OPO-08 primers. Lane X- Standard DNA marker, Lanes: G1 (Ijebu-Ode); G2 (Obafemi- Owode); G3 (Odeda); G4 (Imeko-Afon); G5 (Ifo); G6 (Abeokuta North); Y1 (Ogbomosho North); Y2 (Ogbomosho South); Y3(Ona-Ara); Y4 (Egbeda); Y5 (Ido); S1 (Ejigbo); S2 (Iwo); S3 (Ife-North); S4 (Ilesha-East); L1 (Surulere); L2 (Ikeja); L3 (Ifako ijaye); L4 (Agege); M (Mali).

The similarity matrix ranged from 0.039-1.188 in Table 4. The highest similarity index, of 1.188 was observed between accessions L-04 (Agege) and S-03 (Ife-North), while the least similarity index, (0.0039) was observed between S-02 (Ife-North) and G-03 (Odeda); Y-02 (Ogbomosho-South) and S-02 (Iwo); Y- 05 (Ido) and Y-03 (Ona-Ara) as well as between L-02 (Ikeja) and Y-05 (Ido). From the similarity matrix, it was shown that accession S-03 (Ife-North) was the most distant from all others in all combinations except for the distance between accession L-04 (Agege) and accession G-04 (Imeko-Afon) with similarity index of 0.824 compared with accessions S-03 (Ife-North) and G-04 (Imeko-Afon) with similarity index of 0.768. The findings from this study show that S-03 is an out-group among other accessions. Similar coefficient index which ranged from 0.01 to 0.82 indicated genetic variation. The dendogram established phylogenetic relationship among the accessions from diverse locations as previously observed.

The amplification profiles for specific oligonucleotide primers in RAPD-PCR assays are greatly dependent on specific conditions of reactions; banding patterns thus, vary extensively due to inconsistencies in number of reaction parameters. The fully optimised, RAPD-PCRs could be reliable, cost-efficient molecular methodology in phylogenetic studies. RAPD has been used to analyse genetic variation in several species [24,25] and RAPD-based linkage maps had been reported [26].

Banding pattern of the three RAPD primers; OPO-01, OPO-06 and OPO-08 showing a total 242 scorable bands with mean polymorphic bands of 92.30% and polymorphic information content (PIC) of 0.86 revealed high polymorphism in *Ocimum gratissimum*, which could be due to the genetic variation and nature of RAPD (Random Amplified Polymorphic DNA) marker in accordance with an earlier findings [27].

Furthermore, cluster analysis of the molecular data which was grouped into three clusters revealed that cluster grouping was not related to geographical background of the accessions. Accession from Mali was grouped with accessions from Oyo (Y-03, Y-04and Y-05) and Lagos (L-01, L-02 and L-03), whereas accession L-04 from Lagos was not grouped, but supported the observation of Matasyoh [12]. Virangama and Goyal [28] also reported that dendrogram indicate that cluster groups consisted of accessions from different geographical background though, geographical diversity may not necessarily be associated with genetic diversity. However, the Similarity matrix among the nineteen accessions of *Ocimum gratissimum* which ranged from 0.039-1.188 shows that accession S-03 (Ife-North) was the most distant from other accessions which indicated that it is an out-group among all the accessions. More so, at the state level, it was distinctly shown that accessions from the same state could be distanced genetically from other accessions from different states as observed in cluster II which comprised of two states (Oyo and Lagos) and Mali. Invariably, cluster III also comprised of accessions from Oyo, Ogun and Osun. Moreover, little preference was given to states of collection because some accessions within the same state strived to show similarity by falling into the same cluster group as observed in G-02 and G-04 as well as Y-03 and Y-05. This also supported the findings who reported that accessions from the same geographic region were found in different clusters [29,30]. This could be attributed to similarity in requirements, heterogeneity, population genetic architecture, selection history and approach under domestic cultivation and developmental traits [31,32].

However, the difference at molecular level can be explained based on their long span of cultivation in different areas which differ in soil types, climatic conditions and cultivation practices [33]. The study reveals a wide range of genetic differences which is an indication of genomic variation and relationship among different accessions with broad genetic base.

4. CONCLUSION AND RECOMMENDA-TION

The Random Amplified Polymorphic DNA (RAPD) primers considered in the study were polymorphic for the accessions except G-06 from Mile 2. Accession Y3 from Ona-Ara which yielded the highest total volume of DNA concentration and genomic DNA concentration for accession L4 from Agege could be improved for future breeding. OPO-08 and OPO-06 primers which produced the highest polymorphism and number of polymorphic bands could be recommended for further molecular diversity studies. Cluster II recorded the highest with seven accessions, while S3 from Ile-Ife was the most distant with highest similarity index. Therefore, RAPD could be useful in discrimination, characterization and

differentiation of *O. gratissimum* accessions into groups based on similarity index. However, this study could be indices for the selection of promising accessions, characters and primers of *O. gratissimum* in order to provide information for gene bank data base resources.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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