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GENETIC AND MOLECULAR RELATIONSHIP AMONG CUCUMEROPSIS MANNII NAUDIN USING RAPD MARKER

Olawuyi Odunayo Joseph[1+]
Adedeji Iyanu[2]

[1]*Department of Botany, Genetics and Molecular Biology Units, University of Ibadan, Ibadan, Nigeria.*
Email: olawuyiodunayo@yahoo.com Tel: 08037638364
[2]*Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.*
Email: iyanu.adedeji@aaua.edu.ng Tel: 08131896460


(+ Corresponding author)

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ABSTRACT

Twenty-four accessions of Cucumeropsis mannii obtained from National Centre for Genetic Resources and Biotechnology and some markets from Seven Local Governments in Ibadan were evaluated for genetic relationship using RAPD marker. The field experiment was conducted at the research farm of the Department of Botany, University of Ibadan and arranged in a Completely Randomized Design with four replicates. The molecular experiment was done at International Institute of Tropical Agriculture, Ibadan. The mean square interaction of accessions and their Local governments were significantly (P<0.01) higher for growth, agronomic and yield characters. Accession C3 from Mokola performed best in morphological and yield characters while NGB/01611, NG/TOLO2/11/150 and NG/AA/03/11/040 had the least. Plant height is positively associated with number of leaves (r=0.73), leaf width(r=0.72), number of fruits (r=0.52) and leaf length (0.51). The number of leaves is positively related with leaf width, dry shoot weight, dry leaf weight and dry root weight at r= 0.94, 0.74, 0.73, 0.63 respectively, while dry root weight and fruit weight (r=0.73) are positively related. Two cluster groups were delineated from twenty-four accessions of C. mannii in the dendogram. Cluster 1 constitutes the largest 17 cluster groups and different sub-groups. Accession C12 from Lalupon had highest genomic DNA concentration of 1.89 ug, while highest total volume of 827.9 ul was recorded for C15 from Olodo. Primer OPHO9 had highest allele diversity and polymorphic information content at 0.93 and 92.23% respectively. Therefore, variability among accessions of C. mannii could promote the conservation of C. mannii for genetic improvement program.

Contribution/Originality: This study is one of the few studies which investigated diversity of Cucumeropsis, and has contributed to the existing literature. However, this study revealed that there is existence of variability among the accessions of Cucumeropsis mannnii at the morphological level and molecular level using RAPD marker.

1. INTRODUCTION

The Egusi melon (*Cucumeropsis mannii* Naudin) belongs to the family Cucurbitaceae. It is extensively grown for food in the seeds in West Africa, particularly in Nigeria, Ghana and Togo (Achigan-Dako, Vodouche, & Sangare, 2008; Egunjobi & Adebisi, 2004; Messiaen, 1992). In most Nigerian diets, it is a popular vegetable because of its valuable oil extracted while the plant seed is used to cook various delicacies, such as cake and soup (Denton & Olufolaji, 2000). The quality and quantity of oil derived from the seed vary according to cultivars (Adewusi,

Ladipo, Sarumi, Adebisi, & Vodouhe, 2000). It comprises 34-38% undefatted and 69-78% defatted protein, 11% starch, 2.50% soluble sugars and 12% crude fibre and ash (Fayemi, 1999). The genetic diversity of this plant family and its wide tolerance in tropical and subtropical environments, arid deserts and temperate locations are well established. Details on genetic characterization had been generated between melon and other vegetable crop accessions using morphological and molecular markers to link the relationship among melon and other vegetable crop accessions (Bamigbegbin, Olawuyi, & Jonathan, 2016; Staub, Fanourakis, & López-Sesé, 2004; Stepansky, Kovalski, & Perl-Treves, 1999). Since morphological characteristics are influenced by various environmental factors and pleitrophic gene effect, morphological markers are not accurate to provide exact diversity information (Andersen & Lübberstedt, 2003; Bello & Olawuyi, 2015). Molecular markers are more useful for study in the field of diversity and have proved effective in determining genetic characterization and clarifying genetic connections between and within plants species (Gostimsky, Kokaeva, & Konovalov, 2005; Gupta, Varshney, Sharma, & Ramesh, 1998). Despite the socio-economic, cultural, agronomic and culinary importance of *Cucumeropsis mannii*, there had been low yield of its production. Hence, there is need to characterize *C. mannii* with a view to improving its germplasm conservation for utilization. This study aimed at characterizing *Cucumeropsis mannii* phenotypically and establishing molecular relationship among the accessions using RAPD marker.

2. MATERIALS AND METHODS

2.1. Experimental Site and Seeds Collection

An open field experiment was conducted from the period of December, 2016 to March, 2017 at the nursery farm of the Department of Botany, University of Ibadan, Nigeria. Located in the rainforest Area of Southwestern Nigeria between Latitude 7°02' 49" and 7°43′ 21" N longitude 3°31′ 58" and 4°08′ 20" E with an altitude of 150 m in the valley at 275 m above sea level at moderate annual rainfall of 1,205 mm. The molecular experiment was carried out in Biosciences laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. Twenty –one accessions of *Cucumeropsis mannii* were collected from three selected markets in each of the Seven Local Governments in Ibadan, while three varieties were obtained from the gene bank of National Centre for Genetic Resources and Biotechnology Ibadan. Three varieties of *C. mannii* obtained from the gene bank of National Centre for Genetic Resources and Biotechnology (NACGRAB) were: NGB01611, NG/TOLO2/11/150 and NG/AA/03/11/040, while the markets accessions; CI-C3 (Ibadan-North), C4-C6 (Ibadan South-west), C7-C9 (Akinyele), C10-C12 (Lagelu), C13-C15 (Ido), C16-C18 (Ona –Ara) and C19-C21 (Oluyole) Local Governments.

2.2. Experimental Design, Plant Spacing and Planting Method

The field experiment was a Complete Randomized Design (CRD) with a total of 96 perforated polythene pots each filled with 5 kg of loamy soil and arranged in four replicates. Three seeds were sown in each pot along a row with 1.0 m spacing within the rows and column. The seedlings were thinned to one after three weeks of planting. Agronomic practices were carried out according to the standard procedures.

2.3. Harvesting of Plant Leaves and DNA Extraction

A total of three weeks old seventy–two fresh leaves of *Cucumeropsis mannii* were harvested in an ice bag and lypholised at -80 ^{0}C. Modified Dellaporta DNA extraction protocol was adopted. 200 mg of fresh young leaves of *C. mannii* accessions were grinded with mortar and pestle using liquid nitrogen followed by the addition of 700 ul of pre-heated extraction buffer in 1.5 ml eppendorf tube. The cellular constituents were digested using detergents such as Sodium dodecyl Sulphate (SDS), Cetyl Trimethyl Ammonium Bromide (CTAB) for the removal of membrane lipids. When the DNA is released, it is protected from endogenous nucleases by the inclusion of EDTA in the extraction buffer for the chelating magnesium ions that are significant co-factor for nucleases. The eppendorf tubes

were mixed by occasionally inverting the tubes to homogenize the incubated samples at 65 °C for 20 minutes in water bath. The tubes were removed and allowed to cool for 2 minutes.

500 ul of ice-cold 5M potassium acetate (PH 5.5) was added to each of the tubes and incubated (4°C) on ice for 20 minutes to precipitate the protein. The mixture was centrifuged at 12000 rpm for 10 minutes and 500 ul of ice-cold isopropanol was added to the supernatant, gently mixed and kept for incubation at -80 °C for 15 minutes. The solution was centrifuged at 12000 rpm for 10 minutes to precipitate the DNA, and the pellet was dissolved in double sterile distilled water. The DNA extract usually contains sizeable amount of RNA, proteins, polysaccharides, tannins and pigments that may interfere with the extracted DNA. An addition of protein degrading enzyme known as proteinase-K, was used to remove the protein, while RNase was used to remove RNA, followed by denaturation at 65 °C and precipitation using chloroform and Isoamyl alcohol. The DNA solutions was transferred to 2 ml eppendorf tube and treated with 2 ul of RNase for 40 minutes at 37 °C. 700 ul of Chloroform isoamyl alcohol (24:1) was added and gently mixed to further precipitate protein and lipids. The solution was centrifuged at 12000 rpm for 10 minutes. Since, polysaccharide contaminants are more tasking to remove the combinations of NaCl and CTAB had been observed to remove polysaccharides contaminants (Murray & Thompson, 1980; Paterson, Brubaker, & Wendel, 1993). Moreover, some protocols replaced NaCl with KCl (Thomson & Henry, 1995). Since DNA will be released along with other compounds like lipids, proteins, carbohydrates, or phenols. It needs to be seperated from other compounds by centrifugation. The DNA in the aqueous phase was then transferred into new eppendorf tube without disturbing the interphase and ice-cold ethanol was added to precipitate the DNA in salt solution (e.g Sodium acetate) or alcohol (100% isopropanol or ethanol or ethanol), re-dissolved in sterile water or buffer. The precipitate was centrifuged at 12000 rpm for 10 minutes and the supernatant discarded. The pellet was washed in 70% ethanol, air dried and finally dissolved 60ul of sterile double distilled water.

2.4. Gel Electrophoresis and DNA Purification

The determination of DNA concentration extracted was measured using 1% agarose gel electrophoresis and detected using UV illuminator or spectrophotomer.

Agarose gel checks whether the DNA is degraded or not but estimating DNA concentration by visually comparing band intensities of the extracted DNA with a molecular ladder of known concentration is too subjective. Purity of DNA in the samples, disolved in TE buffer was analysed by checking the intensity of DNA absorbance ratio at 260/280 nm on nanodrop spectrophotometer followed by determination of concentration.

2.5. Polymerase Chain Reaction (PCR), Amplification Conditions and Amplification of DNA Product

PCR reactions for Random Amplfied Polymorphic DNA (RAPD) was performed in the presence of forward and reverse primers that annealled at the 5^1 and 3^1ends of the template DNA respectively. PCR fragments were usually seperated on 3% agarose gels using Ethidium bromide staining reagent, which can be used when differences in allele size among samples is larger than 10 base pairs.Amplification of the loci were carried out in 10 ul PCR reactions containing 3.0 ul of 100 ng/ul total genomic DNA, 2.5 ul of 10x PCR buffer, 1 ul of 25 mM $MgCl_2$, 1 ul of primers each, 1 ul of DMSO, 2 ul of 2.5 mM DNTPs, 1 ul of Taq DNA polymerase using Master Mix. The total reaction volume was made up to 25 ul using 14.4 ul nuclease free water. The amplification conditions were: Initial denaturation at 94°C for 5 mins, followed by 40 cycles of denaturation at 94 °C for 30 s, an annealing step of 30 s at 37 °C and an extension step at 72 °C for 1min. Final extension step at 72 °C for 7 minutes to ensure the completion of the primer extension, followed by hold temperature at 10 °C lasting for infinity. Amplified fragments were visualized on agarose electrophoresis gels stained with 1.5% ethidium bromide.

2.6. Determination of Growth, Agronomic and Yield Characters

Data collection on growth characters of *Cucumeropsis mannii* cultivars commenced at 2 weeks after sowing

(WAS) and was done every two weeks till the 12th week after planting. Data collected were: Leaf width, leaf length, plant height, number of leaves, number of days to flowering, number of fruits and number of flowers per plant. Harvesting of fruits was done at the fifteenth week after planting on the field. Data were collected on yield related characters of the cultivar which includes; Dry leaf weight (g), dry shoot weight (g), dry root weight (g) and fruit weight (g). The Genotypic variance, phenotypic variance and heritability were determined.

2.7. Data Analysis

Morphological data obtained were subjected to analysis of variance (ANOVA). Means were separated using Duncan's Multiple Range Test (DMRT), while the relationships among the growth and yield related characters were established using Dendogram, Correlation co-efficient and principal component analysis (PCA). Molecular data was statistically analysed in order to generate information on total gene diversity, Power marker V3.25 (Liu & Muse, 2005). These values were used to generate dendogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA) cluster analysis as described by Sneath and Sokal (1973) to reveal phenetic representation of genetic relationship among melon accessions.

3. RESULTS

3.1. Mean Square Effect on Growth, Agronomic and Yield Characters of Cucumeropsis Mannii

The result of the mean square effect of accessions in Table 1 showed that highly significant effect ($p<0.01$) was produced on plant height, number of leaves and number of fruits, while leaf width and number of flowers characters had similar effect on accessions.

3.2. Mean Square Interaction of Accessions and Local Governments on Growth, Agronomic and Yield Characters at Different Stages in Cucumeropsis Mannii

The result in Table 2 shows that Local government (LG), Weeks after planting (WAP), first order of interaction (LG x Accessions) and second order of interaction (LG x Accessions x Replicate (Rep)) had highly significant effect ($P<0.01$) on plant height, while Accessions, first order of interaction (LG x Rep, LG x WAP and Accessions x Replicate) were significant ($p<0.05$). Also, LG, WAP, first order of interaction (WAP x Rep) produced highly significant effect on leaf length, while first order of interaction (LG x Rep, LG x WAP and LG x Accessions) and second order of interaction (LG x Location x REP) were significant. The effect of Weeks was highly significant for leaf width, while Local government, first order of interaction (LG x WAP, LG x Accessions and Accessions x Rep) and second order of interaction (Accessions x WAP x Rep) produced significant effect. Local government, Weeks and first order of interaction; (Accessions x Rep) were highly significant for number of leaves while first order of interaction; (LG x WAP) and (LG x Accessions) and second order of interaction (LG x Accessions x Rep) produced significant effect. The number of fruits had highly significant effect for Local government and Weeks, while first order of interaction; (LG x Rep, LG x Week and LG x Accessions) and second order of interaction (LG x Accessions x Rep) were significant. The effect of number of flower was significant on Local government, Weeks, first order of interaction (Week x Rep), while first order of interactions; (LG x Week, LG x Accessions and Accessions x Rep) and second order of interaction (LG x Accessions x Rep and LG x Accessions x Week) were significant. Local government, Weeks, Accessions, first order of interaction (LG x Week, LG x Accessions, Accessions x Rep and Accessions x Week) and second order of interaction (LG x Accessions x Week and Accessions x Weeks x Rep) were highly significant for dry root weight, while first order of interaction (Week x Rep) and second order of interaction (LG x Week x Rep) produced significant effect. The effect of the Weeks on dry leaf weight was highly significant, while Accessions, first order of interaction (Accessions x Rep), (Accessions x Week) and second order of interaction (Accessions x Week x Rep) produced significant effect. Also, the effect of week on dry shoot weight was highly significant, while first order of interaction (LG x Week) and (Accessions x Replicate)

as well as second order of interaction (Accessions x Week x Rep) were significant. The first order of interaction (Week x Rep) and Week had significant effect on the fruit weight.

3.3. Growth, Agronomic and Yield Characters of Cucumeropsis Mannii Accessions

The Performance of growth, agronomic and yield characters of *C. mannii* accessions from different locations revealed significant ($p<0.05$) differences in Table 3. Accession C3 (Mokola) recorded the highest mean leaf length (7.28 cm), leaf width (32.09 cm), number of leaves (40.41), number of flowers (5.94), dry root weight (0.20 g), dry leaf weight (2.48 g) and dry shoot weight (5.0 g), while Accession C2 (Orita-merin), had the highest plant height (83.70 cm) and number of fruits (0.63). The plant height of accessions; C5 (Oke Ado), C7 (Ojoo), C8 (Moniya), C9 (Ijaye), C12 (Lalupon), C15 (Omi), C19 (Orita-Challenge), C20 (Odo Ona), NGB/01611 and NG/AA/03/11/040 as well as accession C4 (Bode), C11(Olodo) and accession C6 (Oja Oba), C17 (Oluloyo), and NG/TOLO2/11/150 are not significantly different. The leaf length of accessions; C6 (Oja Oba), C12 (Lalupon), C18 (Amuloko) and NG/TOLO2/11/150 as well as number of leaves in accessions; C6 (Oja Oba), C13 (Apete), C17 (Oluloyo), C18 (Amuloko) and NG/TOLO2/11/150 l are not significantly different from one another.

Also, the number of fruits, in accession C7 (Ojoo), C9 (Ijaye), C16 (Olorunsogo) and C21 (Idi Ayunre) as well as C4 (Bode), C12 (Lalupon), C17 (Oluloyo), C19 (Amuloko), C20 (Odo-ona) and NG/AA/03/11/040; and accessions C5 (Oke ado) and NG/TOLO2/11/150 were statistically similar. The Number of flowers in accessions; C6 (Oja Oba), C20 (Odo Ona) and NG/AA/03/11/040 as well as accession C9 (Ijaye) and C10 (Lagelu) were also similar. The dry root weight of accessions; C1 (Bodija,) C2 (Orita-merin), C4 (Bode), C7 (Ojoo), C9 (Ijaye), C11 (Olodo), C14 (Apata), C15 (Omi), C19 (Orita Challenge) and C21 (Idi Ayunre) were not significantly different. The dry shoot weight and dry leaf weight in accession C3 (Mokola) were significantly different from other accessions.

3.4. Growth, Agronomic and Yield Characters of Cucumeropsis mannii Based on Local Governments and NACGRAB.

The result in Table 4 also showed the performance of growth, agronomic and yield characters of *C. mannii* based on Local governments and NACGRAB. It reveals that accessions from Ibadan North Local government is significantly higher ($p<0.05$) for plant height (77.20 cm), leaf length (6.94), leaf width (20.84 cm), number of leaves (25.91), number of fruits (0.83), number of flowers (3.94), dry root weight (0.12 g), dry shoot weight (2.11 g) and dry leaf weight (0.97 g). The mean of Plant height in Ibadan South-West, Akinyele, Ido, Ona Ara, Oluyole Local Governments and NACGRAB were not significantly different from one another. Also, leaf length in Ibadan South-West, Lagelu, Ido, Ona Ara, Oluyole Local Governments and NACGRAB were significantly similar. The leaf width for Ibadan South-west, Ido, Oluyole Local governments were not significantly different. The Number of leaves mean for Ibadan South-West, Oluyole Local governments and NACGRAB were not significantly different. The mean number of fruits for Ido, Ona Ara, Oluyole Local governments and NACGRAB were not significantly different while the mean values of number of flowers in Ido and Oluyole Local Government as well as Ona Ara Local Government and NACGRAB were not significantly different. The mean value of dry root weight in Lagelu and Ido Local Governments as well as Ibadan South-West and NACGRAB were not significantly different. The mean values of dry shoot and dry root weights in Lagelu, Ido, Ona Ara, Oluyole Local governments and NACGRAB were not significantly different.

3.5. Phenotypic Variance, Genotypic Variance and Heritability Estimates of Growth, Agronomic and Yield Characters in Cucumeropsis Mannii.

The result in Table 5 showed that the phenotypic variance of both growth and yield characters were higher than the genotypic variance in all the characters. The highest values of phenotypic (1320.56), genotypic (874.10) variances and heritability estimates were recorded in plant height.

3.6. Principal Component Analysis (PCA) of Growth, Agronomic and Yield Characters of Cucumeropsis mannii.

The fourteen (14) principal component axes were extracted for all the evaluated characters out of which the first three with the eigen value greater than 1 accounted for 76.12% of the total variation. Table 6. The first Principal Component axis accounted for 47.89%. Characters such as t Prin 1 (47.89%), Prin 2 (15.65%) and Prin 3 (12.58%). The Prin 1 accounted for the highest proportion of 47.89% of eigen value of 4.80 of the total variation. It was observed that dry shoot weight (0.42), dry leaf weight (0.42), number of leaves (0.41) and leaf width (0.41) were closely related characters and had the highest loadings with positive contributions towards the entire variation while number of fruits (0.28), plant height (0.27), number of flowers (0.17), leaf length (0.17) and fruit weight (0.12) were closely related. Characters that contributed more strongly to PC2, which accounted for 15.65% of the total variation, were dominated by traits suchfruit weight (0.68), dry root weight (0.48) while plant height (-0.31), number of flowers (-0.27), number of fruits (-0.27) and number of leaves (-0.21) are closely associated to one another.

3.7. Correlation Co-Efficient among Growth, Agronomic and Yield Characters in Cucumeropsis mannii.

The correlation co-efficient result in Table 7 showed that the plant height is positive and significantly related with number of leaves (r= 0.73), leaf width (r= 0.72) , number of fruits (r= 0.52) and leaf length (r= 0.51) while the number of leaves had a strong positive correlation with leaf width (r= 0.94), dry shoot weight (r= 0.74), dry leaf weight (r= 0.73) and dry root weight (r= 0.63). Also, Leaf width is positive and strongly associated with dry shoot weight (r= 0.74), dry leaf weight (r= 0.72) and dry root weight (r= 0.68). Number of fruits had a correlation positive with dry shoot weight (r= 0.55) and dry leaf weight (r= 0.54). Dry shoot weight had a strong and positive correlation with dry leaf weight (r= 0.99) and dry root weight (r= 0.79) while dry root weight is strongly positive and correlated with fruit weight (r= 0.73). Dry leaf weight is significantly related with dry root weight (r= 0.75).

3.8. Nanodrop Showing the DNA Concentration of Cucumeropsis mannii Accessions

The nanodrop and DNA concentration of the extracted *Cucumeropsis mannii* accessions at 260/280gl as shown in Table 8. The genomic DNA gel result showing the DNA PCR Amplification of *Cucumeropsis mannii* accessions is presented in Figure 11. The Agarose gel electrophoresis of *Cucumeropsis mannii* accessions generated from primers; OPB02, OPB10, OPB12, OPB05, OPH09, OPT01, OPT07, OPT20, OPH06 and OPT06 are shown in Figures 1-10.

The highest total volume of genomic DNA of 827.9/ug was recorded for accession C11 from Olodo. Accession C12 from Lalupon had the highest genomic DNA concentration of 1.89/ug, followed by accession C7 from Ojoo and C21 from Idi-ayunre with the value of 1.88/ug while accession C16 from Olorunsogo had the least (1.09/ug).

3.9. Polymorphic Information Content (PIC), Frequency and Diversity of Allele of Cucumeropsis mannii Using Ten RAPD Primers.

A total of ten primers of RAPD revealed major allele frequency, number of alleles, allele diversity and polymorphic information contents of twenty four accessions of *Cucumeropsis mannii* Table 9. The result showed that Primer OPT07 had the highest major allele frequency of 0.42, while Primers OPH05 and OPH09 had the lowest major allele frequency of 0.13, Primer OPB10, OPB12 and OPT20 had the same major allele frequency of 0.25, while Primer OPT01 and OPH06 had 0.29. Primer OPB12 had the highest number of allele at 18 while Primer OPT07 had the least (6), Primers OPB10, OPH05, OPH09 and OPH06 had the same number of 16 alleles. Primer OPH09 had the highest value of allele diversity and polymorphic information content at 0.93 and 92.23% respectively.

Table-1. Mean square effect on growth, agronomic and yield characters of *Cucumeropsis mannii* accessions.

Source of variation	Df	Plant height	Leaf length	Leaf width	Number of leaves	Number of fruits	Number of flower	Dry root weight	Dry shoot weight	Dry leaf weight	Fruit weight
Accessions	23	4982.09***	10.40^{ns}	602.43*	785.22***	0.80***	29.47*	0.03^{ns}	22.68^{ns}	5.86^{ns}	7.89^{ns}
Error	486	257.48	8.26	324.25	257.48	0.24	17.68	0.04	17.8	4.43	1.12
Corrected Total	509										

Note: * = Significant at P<0.05.
** = highly significant at P<0.01.
*** = highly significant at P<0.001, ns = non-significant, Df = degree of Freedom.

Table-2. Mean square interaction of accessions, local government on growth, agronomic and yield characters at different stages in *Cucumeropsis mannii*.

Source of variation	Df	Plant height	Leaf length	Leaf width	Number of leaves	Number of fruits	Number of flowers	Dry root weight	Dry leaf weight	Dry shoot weight	Fruit weight
Local government (LG)	7	9073.54***	10.40***	566.07**	983.93***	1.90***	59.87***	0.05***	4.66^{ns}	21.04^{ns}	0
Replicates	3	390.46	10.99	196.66	175.16	0.94	78.35	0.02	5.10	25.04	6.67
Weeks	5	43500.84***	293.44***	9417.48***	5293.00***	2.15***	476.61***	1.77***	43.87***	240.63***	100.00***
Accessions	2	3942.86**	4.20^{ns}	116.13^{ns}	171.41^{ns}	0.24^{ns}	4.36^{ns}	0.08***	13.21*	36.52^{ns}	0^{ns}
LG x Replicate	21	1055.50*	3.90**	121.13^{ns}	107.99^{ns}	0.38*	4.17^{ns}	1.01^{ns}	3.03^{ns}	10.94^{ns}	0^{ns}
LG x Week	35	1039.50**	2.73*	216.05*	191.03*	0.42**	17.92*	0.03***	4.12^{ns}	18.37*	0^{ns}
LG x Accessions	14	1752.65***	3.87*	293.30*	348.68*	0.34*	17.86*	0.03***	4.65^{ns}	20.73^{ns}	0^{ns}
Week x Replicate	15	615.22^{ns}	56.63***	152.64^{ns}	142.74^{ns}	0.31^{ns}	137.19***	0.01*	2.85^{ns}	13.43^{ns}	6.67***
Accessions x Replicate	6	1421.72*	1.67^{ns}	525.83**	640.67***	0.09^{ns}	25.25*	0.04***	8.07*	31.36*	0.00^{ns}
Accessions x Week	10	590.79^{ns}	1.28^{ns}	116.49^{ns}	109.53^{ns}	0.14^{ns}	7.38^{ns}	0.04***	7.20*	19.91^{ns}	0.00^{ns}
LG x Week x Replicate	99	310.45^{ns}	1.86^{ns}	87.16^{ns}	95.81ns	0.16^{ns}	8.86^{ns}	0.01*	3.11^{ns}	11.19^{ns}	0.00^{ns}
LG x Accessions x Replicate	42	1509.10***	2.47*	161.14^{ns}	237.48*	0.41**	14.1*	0.01^{ns}	2.87^{ns}	30.82^{ns}	0.00^{ns}
LG x Accessions x Week	70	352.83^{ns}	1.31^{ns}	157.61^{ns}	161.50^{ns}	0.15^{ns}	10.49*	0.02***	4.13^{ns}	14.58*	0.00^{ns}
Accessions x Week x Replicate	30	283.99^{ns}	1.29^{ns}	268.33*	219.10*	0.19^{ns}	9.82^{ns}	0.02***	4.91*	18.45*	0.00^{ns}
Corrected Total	509										
Error	150	446.46	1.4	129.99	127.46	0.19	7.57	0.01	13.21	12.09	0.00

Note: * = Significant at P<0.05.
** = highly significant at P<0.01.
*** = highly significant at P<0.001, ns = non-significant, Df = degree of freedom.

Table-3a. Growth, agronomic and yield characters of *Cucumeropsis mannii* accessions.

Accessions/ Locations	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Number of fruits	Number of flower	Dry root weight (g)	Dry shoot weight (g)	Dry leaf weight (g)	Fruit weight (g)
C1-Bodija	65.53^{abc}	6.27^{ab}	13.64^{b}	20.07^{bc}	0.46^{abc}	3.57^{abcde}	0.08^{ab}	0.86^{b}	0.24^{b}	0.42^{a}
C2-Orita-	83.70^{a}	7.25^{a}	6.94^{b}	26.24^{b}	0.63^{a}	4.92^{ab}	0.07^{ab}	0.45^{b}	0.18^{b}	0.42^{a}
C3-Mokola	82.37^{ab}	7.28^{a}	32.09^{a}	40.41^{a}	0.54^{a}	5.94^{a}	0.20^{a}	5.01^{a}	2.48^{a}	0.42^{a}
C4-Bode	52.65^{ced}	5.98^{ab}	14.91^{b}	20.37^{bc}	0.04^{e}	3.78^{abcde}	0.08^{ab}	0.60^{b}	0.30^{b}	0.42^{a}
C5-Oke-Ado	45.04^{cedf}	5.45^{ab}	11.10^{b}	17.46^{bc}	0.08^{de}	3.35^{abcde}	0.02^{b}	0.28^{b}	0.18^{b}	0.42^{a}
C6-Oja-Oba	29.40^{ef}	4.84^{b}	7.13^{b}	12.34^{c}	0.13^{cde}	2.15^{bcde}	0.03^{b}	0.20^{b}	0.07^{b}	0.42^{a}
C7-Ojoo	39.63^{cedf}	6.25^{ab}	14.44^{b}	18.21^{bc}	0.21^{bcde}	3.83^{abcde}	0.10^{ab}	0.92^{b}	0.41^{b}	0.42^{a}
C8-Moniya	44.19^{cedf}	6.29^{ab}	14.54^{b}	19.98^{bc}	0.50^{ab}	4.69^{abc}	0.05^{b}	0.60^{b}	0.28^{b}	0.42^{a}
C9-Ijaye	45.66^{cedf}	6.42^{ab}	17.43^{b}	22.79^{bc}	0.21^{bcde}	4.63^{abcd}	0.11^{ab}	1.69^{b}	0.91^{b}	0.42^{a}
C10-Lagelu	61.85^{abcd}	6.26^{ab}	13.53^{b}	19.52^{bc}	0.13^{cde}	4.60^{abcd}	0.06^{ab}	0.48^{b}	0.17^{b}	0.42^{a}
C11-Olodo	55.75^{cde}	5.85^{ab}	16.05^{b}	21.95^{bc}	0.42^{abcd}	4.49^{abcde}	0.08^{ab}	0.69^{b}	0.17^{b}	0.42^{a}
C12-Lalupon	39.31^{cdef}	4.93^{b}	9.81^{b}	15.77^{bc}	0.04^{e}	3.14^{abcde}	0.03^{b}	0.17^{b}	0.10^{b}	0.42^{a}

Note: Mean with the same letter in the same column are not significantly different at P ≥ 0.05 according toDuncan Multiple Range Test (DMRT).

Table-3b. Growth, agronomic and yield characters of *Cucumeropsis mannii* accessions (Cont'd).

Accessions	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Number of fruits	Number of □lower	Dry root weight (g)	Dry shoot weight (g)	Dry leaf weight (g)	Fruit weight (g)
C13-Apete	35.34^{def}	5.43^{ab}	9.10^{b}	14.04^{c}	0.04^{e}	3.41^{abcde}	0.03^{b}	0.34^{b}	0.14^{b}	0.42^{a}
C14-Apata	51.69^{cdf}	5.59^{ab}	13.57^{b}	19.51^{bc}	0.13^{cde}	4.06^{abcde}	0.08^{ab}	0.81^{b}	0.29^{b}	0.42^{a}
C15-Omi	43.25^{cdef}	5.55^{ab}	15.95^{b}	20.84^{bc}	0.13^{cde}	4.17^{abcde}	0.07^{ab}	0.59^{b}	0.31^{b}	0.42^{a}
C16-Olorunsogo	58.78^{bcd}	6.13^{ab}	11.54^{b}	16.92^{bc}	0.21^{bcde}	3.50^{abcde}	0.04^{b}	0.35^{b}	0.14^{b}	0.42^{a}
C17-Oluloyo	31.19^{ef}	5.88^{ab}	7.18^{b}	10.98^{c}	0.00^{e}	1.68^{de}	0.04^{b}	0.26^{b}	0.05^{b}	0.42^{a}
C18-Amuloko	24.64^{f}	4.71^{b}	5.91^{b}	10.82^{c}	0.00^{e}	1.58^{e}	0.02^{b}	0.11^{b}	0.01^{b}	0.42^{a}
C19-OritaChallenge	50.57^{cdef}	5.81^{ab}	10.58^{b}	17.97^{bc}	0.18^{cde}	3.72^{abcde}	0.07^{ab}	0.59^{b}	0.19^{b}	0.42^{a}
C20-Odo-Ona	42.45^{cdef}	6.13^{ab}	12.77^{b}	16.69^{bc}	0.00^{e}	2.92^{bcde}	0.05^{b}	0.29^{b}	0.14^{b}	0.42^{a}
C21-Idi Ayunre	36.92^{def}	5.23^{ab}	9.81^{b}	16.18^{bc}	0.21^{bcde}	3.86^{abcde}	0.10^{ab}	0.45^{b}	0.19^{b}	0.42^{a}
NGB/01611	51.29^{cdef}	5.89^{ab}	10.27^{b}	15.99^{bc}	0.18^{cde}	3.38^{abcde}	0.03^{b}	0.27^{b}	0.12^{b}	0.42^{a}
NG/Tolo2/11/150	29.94^{ef}	4.31^{b}	7.49^{b}	13.13^{c}	0.08^{de}	1.84^{cde}	0.05^{b}	0.49^{b}	0.15^{b}	0.42^{a}
NG/AA/03/11/040	38.37^{cdef}	5.94^{ab}	12.25^{b}	18.89^{bc}	0.00^{4e}	1.98^{bcde}	0.05^{b}	0.33^{b}	0.09^{b}	0.42^{a}

Note: Mean with the same letter in the same column are not significantly different at P ≥ 0.05 according toDuncan Multiple Range Test (DMRT).

Table-4. Growth, agronomic and yield characters of *Cucumeropsis mannii* from NACGRAB and Local Governments in Ibadan.

Local Governments	Plant height (cm)	Leaf length(cm)	Leaf width (cm)	Number of leaves	Number of fruits	Number of flower	Dry root weight (g)	Dry shoot weight (g)	Dry leaf weight (g)	Fruit weight (g)
Ibadan-North	77.20^{a}	6.94^{a}	20.84^{a}	25.91^{a}	0.83^{a}	3.94^{a}	0.12^{a}	2.11^{a}	0.97^{a}	O.42^{a}
Ibadan South-West	42.94^{c}	5.45^{c}	11.25^{bcd}	16.92^{bc}	0.54^{ab}	2.25^{c}	0.04^{cd}	0.37^{b}	0.19^{b}	O.42^{a}
Akinyele	43.16^{c}	6.32^{b}	15.47^{b}	20.33^{b}	0.31^{b}	3.35^{ab}	0.08^{b}	1.07^{ab}	0.53^{ab}	O.42^{a}
Lagelu	53.48^{b}	5.75^{c}	13.49^{bc}	19.38^{a}	0.19^{bc}	3.06^{abc}	0.06^{bcd}	0.46^{b}	0.15^{b}	O.42^{a}
Ido	43.78^{c}	5.67^{c}	13.01^{bcd}	18.28^{b}	0.10^{c}	2.92^{bc}	0.06^{bcd}	0.58^{b}	0.25^{b}	O.42^{a}
Ona-Ara	39.26^{c}	5.63^{c}	8.64^{d}	13.08^{c}	0.07^{c}	1.24^{d}	0.03^{d}	0.24^{b}	0.07^{b}	O.42^{a}
Oluyole	43.40^{c}	5.74^{c}	11.12^{bcd}	16.95^{bc}	0.13^{c}	2.43^{bc}	0.07^{bc}	0.44^{b}	0.17^{b}	O.42^{a}
Nacgrab	4.46^{c}	5.42^{c}	9.87^{cd}	16.34^{bc}	0.10^{c}	1.29^{d}	0.04^{cd}	0.36^{b}	0.12^{b}	O.42^{a}

Note: Mean with the same letter in the same column are not significantly at P ≥ 0.05 according to Duncan Multiple Range Test (DMRT).

Table-5. Phenotypic variance, Genotypic variance and Heritability estimates of growth, agronomic and yield characters in *Cucumeropsis mannii*.

Source of Variation	Genotypic Variance (O^2g)	Phenotypic Variance (O^2g)	Heritability (H^2)
Plant height	874.10	1320.56	0.66
Leaf length	0.70	2.10	0.33
Leaf width	-3.47	126.53	-0.03
Number of leaves	10.99	138.45	0.08
Number of fruit	0.01	0.20	0.06
Number of flower	-0.80	6.77	-0.12
Dry root weight	0.02	0.03	0.64
Dry leaf weight	0.00	13.21	0.00
Dry shoot weight	6.11	36.52	0.34

Table-6. Principal Componenet Analysis (PCA) of growth and Yield characters in *Cucumeropsis mannii*.

Characters	Prin 1	Prin 2	Prin 3
Plant height	0.27	-0.31	0.12
Number of leaves	0.41	-0.20	0.11
Leaf length	0.11	-0.10	0.78
Leaf width	0.41	-0.13	0.07
Number of flower	0.17	-0.27	-0.59
Number of fruit	0.28	-0.27	-0.03
Dry shoot weight	0.42	0.16	-0.07
Dry leaf weight	0.42	0.13	-0.06
Dry root weight	0.34	0.48	-0.06
Fruit weight	0.12	0.64	0.03
Eigen value	4.80	1.57	1.26
Proportion (%)	47.89	15.65	12.58

Table-7. Correlation co-efficient of growth, agronomic and yield characters in *cucumeropsis mannii* accessions.

	PH	NL	LL	LW	NFL	NF	DSW	DLW	DRW	FW	LG	L / Lg	L all	Weeks	Rep.
PH															
NL	0.73**	-													
LL	0.51*	0.49	-												
LW	0.72**	0.94**	0.47	-											
NFL	0.39	0.39	-0.10	0.38	-										
NF	0.52*	0.61**	0.26	0.57*	0.30	-									
DSW	0.44	0.74**	0.24	0.74**	0.31	0.55*	-								
DLW	0.49	0.73**	0.23	0.72**	0.31	0.54*	0.99**	-							
DRW	0.50*	0.63**	0.30	0.68**	0.23	0.37	0.79**	0.75**	-						
FW	0.46	0.33	0.30	0.42	0.10	0.11	0.36	0.31	0.73**	-					
LG	-0.21	-0.20	-0.10	-0.16	-0.12	-0.20	-0.09	-0.10	0.07	-0.02	-				
L / Lg	-0.09	0.07	-0.03	0.06	-0.03	-0.01	0.06	0.07	0.04	-0.00	0.01	-			
L all	-0.22	-0.19	-0.10	-0.15	-0.12	-0.20	-0.08	-0.07	-0.07	-0.02	0.99**	0.13	-		
Weeks	0.72	0.55*	0.49	0.63**	0.22	0.32	0.29	0.25	0.52*	0.59*	-0.06	-0.02	-0.06	-	
Replicates	-0.02	-0.05	-0.06	-0.02	0.08	0.10	-0.03	-0.03	0.02	0.18	0.06	-0.02	0.06	0.03	1.00

Note: ** =highly significant at 1% level of probability.
PH: Plant height, **NL:** Number of leaves, **LL:** leaf length, **LW:** Leaf width, **NFL:** Number of flower, **NF:** Number of Fruit, **DSW:** Dry shoot weight, **DLW:** Dry leaf weight, **DRW:** Dry root weight, **FW:** Fruit weight, **LG:** Local government, **L/lg:** Location with respect to local government**, Lall:** All locations, **Rep:** Replicate.

Table-8. Nanodrop showing the DNA concentration of *Cucumeropsis mannii* accessions.

Accessions	Total Volume of DNA Extracted (ul)	Concentration of Genomic DNA at 260/280gl
Bodija	105.2	1.63
Orita-Merin	141.7	1.48
Mokola	50.8	1.83
Bode	231	1.87
Oke-Ado	300	1.74
Oja-Oba	215.5	1.68
Ojoo	273.6	1.88
Moniya	25.5	1.72
Ijaye	738.5	1.59
Lagelu	600.3	1.85
Olodo	827.9	1.76
Lalupon	367.5	1.89
Apete	417.5	1.87
Apata	822	1.84
Omi	38.1	1.16
Olorunsogo	137.1	1.09
Oluloyo	615.2	1.74
Amuloko	277.5	1.79
Orita-Challenge	15.2	1.37
Odo-Ona	478.1	1.66
IDI –Ayunre	600.1	1.88
NGB/01611	575.9	1.81
NG/TOLO2/11/150	542.2	1.84
NG/AA/03/11/040	510.2	1.87

Table-9. Genetic polymorphisms generated from RAPD primers in *Cucumeropsis mannii* accessions.

Marker	Major Allele Diversity	Allele number	Allele Diversity	Polymorphic Information Content (%)
OPB02	0.17	15	0.91	89.92
OPB10	0.25	16	0.89	88.5
OPB12	0.25	18	0.9	89.73
OPH05	0.13	16	0.92	91.85
OPH09	0.13	16	0.93	92.23
OPT01	0.29	9	0.81	78.84
OPT07	0.42	6	0.74	70.42
OPT20	0.25	12	0.86	84.35
0PH06	0.29	16	0.88	87.11
OPT06	0.36	7	0.73	68.8
Mean	0.25	13.1	0.86	0.84

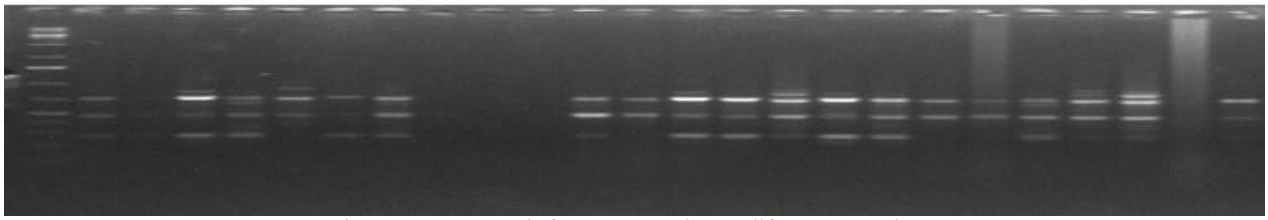

Figure -1. Agarose gel of 24 *Cucumeropsis mannii* for OPBO2 primer.

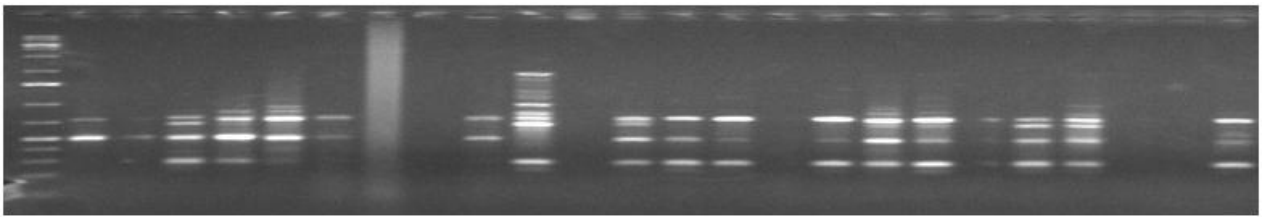

Figure -2. Agarose gel of 24 *Cucumeropsis mannii* for OPB10 primer.

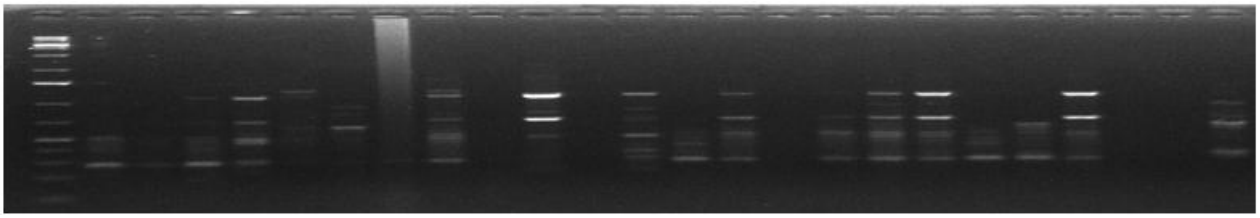

Figure -3. Agarose gel of 24 *Cucumeropsis mannii* for OPB12 primer.

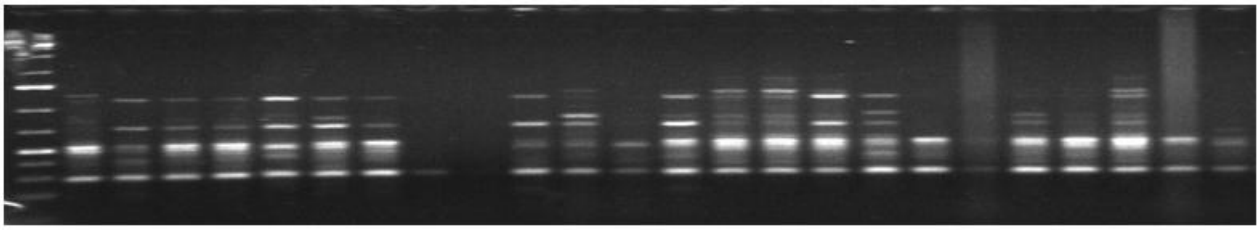

Figure-4. Agarose gel of 24 *Cucumeropsis mannii* for OPH05 primer.

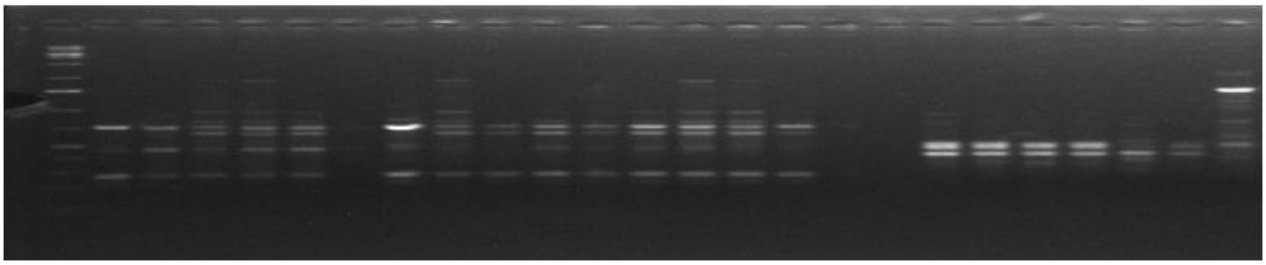

Figure-5. Agarose gel of 24 *Cucumeropsis mannii* for OPH09 primer.

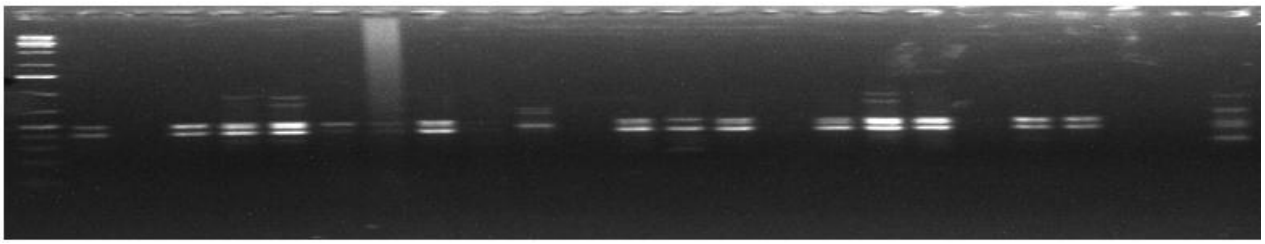

Figure -6. Agarose gel of 24 *Cucumeropsis mannii* for OPT01 primer.

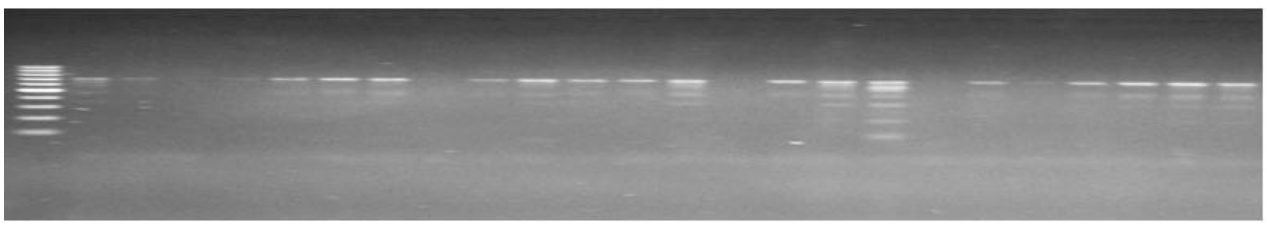

Figure -7. Agarose gel of 24 *Cucumeropsis mannii* for OPT07 primer.

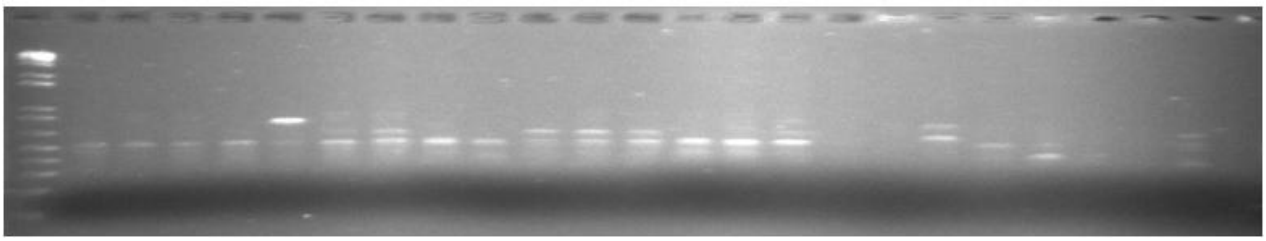

Figure -8. Agarose gel of 24 *Cucumeropsis mannii* for OPT20 primer.

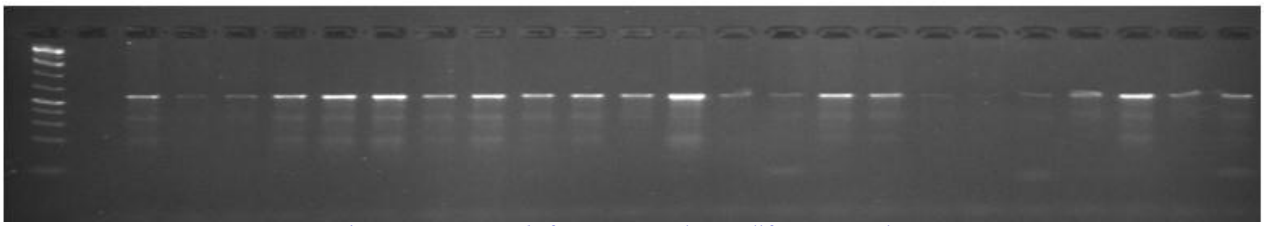

Figure-9. Agarose gel of 24 *Cucumeropsis mannii* for OPT06 primer.

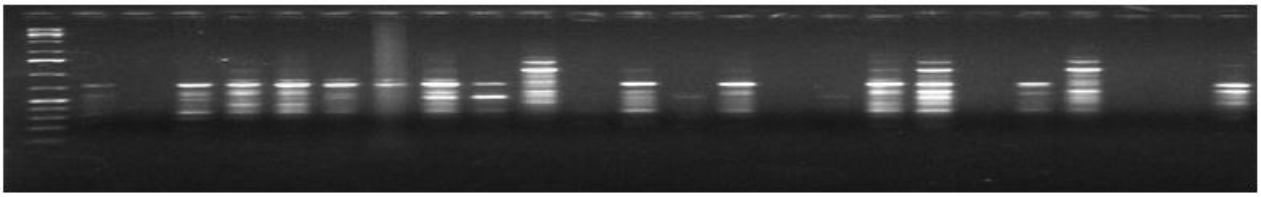

Figure-10. Agarose gel of 24 *Cucumeropsis mannii* for OPH06 primer.

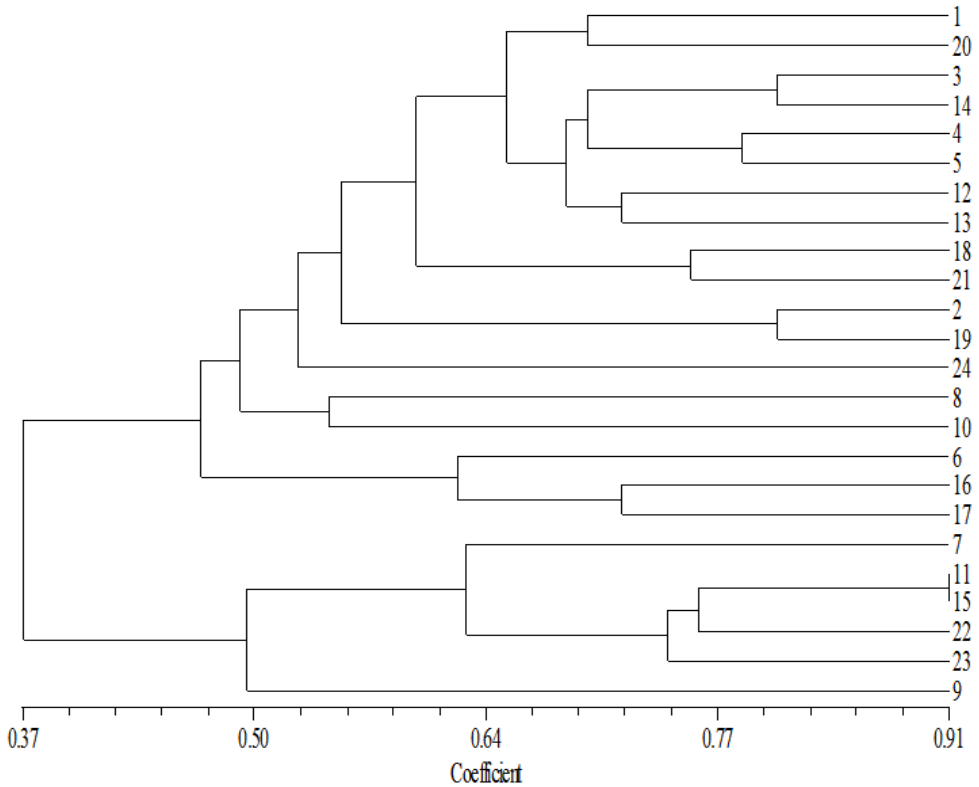

Figure-11. Dendogram showing genetic relationship among 24 accessions of Cucumeropsis mannii.
1(C3-MOKOLA), 20 (C5-OKE-ADO), 3 (C7-OJOO), 14(C8-MONIYA), 4(NG/TOLO2/11/150), 5(C9-IJAYE), 12(C15-OMI), 13(C16-OLORUNSOGO), 18(C11-OLODO), 21(C2-ORITA-MERIN), 2(C13-APETE), 19(C10-LAGELU), 24(C12-OLULOYO), 8(C21- IDI AYUNRE), 10(NG/AA/03/11/040), 6(C1-BODIJA), 16(C20-ODO-ONA), 17(C19-ORITA-CHALLENGE), 7(C18-AMULOKO), 11(C14-APATA), 15(C12-LALUPON), 22(C6-OJA OBA), 23(C4-BODE) and 9(NGB01611).

3.10. Dendogram Showing the Genetic Similarity among Accessions of Cucumeropsis mannii

The dendogram showing the relationship among 24 accessions of *C. mannii* delineated in to two major clusters and sub-clustered in to different groups indicating their level of genetic relationship. The cluster 1 is the largest with seventeen accessions while the cluster 2 comprised seven accessions. Accession C3 from Mokola and C5 from Oke-ado are more closely related compared to accession C8 from Moniya and NG/TOLO2/11/150. Also, accession C9- from Ijaye and C15 from Omi are related but different from C16 from Olorunsogo while accession C11from Olodo and C2 from Orita-merin are similar. Again, accession C13 from Apete and C10 fromLagelu are closely related than accession C17 from Oluloyo while accession C21fromIdi-ayunre and NG/AA/03/040 are related as well as accession C1from Bodija and C20 from Odo-ona. Again, accession C18 from Amuloko and C14 from Apata are more closely related than accession C19 from Orita-challenge likewise accessions C12 from Lalupon and C6 from Oja-oba as well as C4 from Bode and NGB01611 accessions.

4. DISCUSSION

Genetic diversity in vegetative and sexually propagated plants is important in effective breeding programmes. (Olawuyi, Bello, Ntube, & Akanmu, 2015). Genetic variation provides different knowledge on morphological characteristics, genetic intelligence ability and an outstanding base as was affirmed by Upadhyay, Neeraja, Kole, and Singh (2012). From this study, genetic variability observed on growth, agronomic and yield characters of *Cucumeropsis mannii* with respect to the interaction of accessions from market locations in some Local government was in accordance with the findings made by Nwangburuka, Oyekale, Ezekiel, Anokwuru, and Badaru (2012) and Olawuyi, Ezekiel-Adewoyin, Odebode, Aina, and Esebanenm (2012) on some vegetables. The variability is attributed to wide genetic differences and the level of adaptation in the the form (Kulakow, 1987; Mujicia & Jacobsen, 2005).

The accessions from Ibadan-North Local government particularly C3 from Mokola and C2 from Orita-merin performed best for growth, agronomic and yield related characters. The selections based on these characters could thus improve productivity as similarly reported by Adedeji, Ajayi, Osekita, and Ogunruku (2020).

The findings from correlation coefficient shows that plant height had a strong positive correlation with number of leaves, leaf width, leaf length, number of fruits and dry root weight as similarly observed by Olawuyi et al. (2014). The correlation between characters implies that selection based on plant height will favour the growth, agronomic and yield characters. Thus the rate of productivity will be enhanced. Also, the number of leaves which had a strong positive correlation leaf width, number of fruits, dry shoot weight, dry leaf weight and dry root weight will be of the high yield potential.

Prin 1 accounted for the highest variation as previously observed by Olowe, Odebode, Olawuyi, and Akanmu (2013) and Olawuyi et al. (2015). The principal component analysis revealed the trend of variation between characters, which often reflect variability within a group of entries (Aremu, Adebayo, Oyegunle, & Ariyo, 2007). This ensures in particular that prominent characters come together in particular component by adding to variability, which can be used for improving crops.

It was observed that the phenotypic variance of both growth and yield characters evaluated were higher than the genotypic variance. Heritability was higher for only plant height and dry root weight therefore, the proportion of genotypic effect to phenotypic effect was higher for this traits this involved the combination of environmental and genetic factors the high heritability for plant height was in conformity with the work done by Tahernezhad, Saba, Zeinalabedini, Pourdad, and Ghaffari (2018).

Random Amplified Polymorphic DNA (RAPD) which is one of the notable molecular markers, that requires no prior sequence information (Palumbi, 1996). Polymorphism observed in the ten RAPD primers across all the melon accessions accounted for variations in major allele frequency, allele number and allele diversity. This supported the reports of Mallory et al. (2008) and Olawuyi, Kariunwi, and Akanmu (2018).

The relationship that exist among the accessions in the clusters, showed genetic similarities and differences in accordance with the findings made by Bamigbegbin et al. (2016). The delineation of the 24 accessions of *Cucumeropsis mannii* from the dendogram revealed that there are genetic similarities and differences. It was observed that accessions from the same Local Governments were clustered in to different groups Thus, high inter and intra species variability exists even among genotypes (Mosyakin & Robertson, 1996; Stefunova, Bezo, Labajová, & Senková, 2014).

5. CONCLUSION AND RECOMMENDATION

Accession C3 from Mokola performed best on the field for both growth and yield characters, while C12 from Lalupon had the best genomic DNA quality. They could be suggested for improvement of effective germplasm conservation.

OPH09 primer detected the highest polymorphism and allele diversity compared to other primers. This could be recommended for further molecular studies.

The germplasm varieties from NACGRAB; NGB01611, NG/TOLO2/11/150, NG/AA/03/11/04, should be improved for future breeding studies.

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