Original Research Article

MOLECULAR CHARACTERIZATION OF FLUTED PUMPKIN LANDRACES IN SOUTH EAST NIGERIA

ABSTRACT

The fluted pumpkin *Telfairia occidentalis* (Hook F.) is a very important vegetable, popularly cultivated in South East Nigeria for its economic value and its role in nutrition. It exhibits a high degree of interspecies variability and as such enhances biodiversity. Twenty landraces of fluted pumpkin were collected from five States in southeast Nigeria which include; Anambra, Ogun, Lagos, Ondo and Enugu State. The landraces were grown in the Botany Garden of Lagos State University, Ojo during the rainy season of 2013 for phenotypic screening to determine genetic divergence. The experiment was laid out in randomized block design with three replications. Genetic characterization was conducted in the biotech lab of the national institute of medical research (NIMER). The genomic DNA was extracted from the samples and extracted DNA samples were amplified through the PCR techniques using four primers (RAPD – 01, OPR-02, OPC – 04 and SCAR-1 primer) to access diversity among the genotypes. The result reveals a huge genetic divergence among the genotypes studied. The genotypes were grouped into five clusters based on a 2.0(20%) level of similarity by single linkage cluster analysis, which agrees with morphological data, each containing fluted pumpkin genotypes sharing common properties and being similar to one another.

Keywords: variability, genetic divergence, and cluster analysis.

1. INTRODUCTION

BACKGROUND OF STUDY

It is a fact universally accepted and acknowledged that one can achieve long life, fitness and good health through eating healthy diets. Nigeria as a nation grows varieties of vegetables both fruit and leafy. The growing awareness in recent years of the health-promoting and protecting properties has directed attention to vegetables as vital components of daily diets (Smith and Eyzaguirre, 2007). This attention to vegetables reinforces the significant roles of mostly leafy vegetables. The leafy vegetable is an important feature of Nigeria's diet after a traditional meal without it is assumed to be incomplete. In developing countries, the consumption of vegetables is generally lower than the FAO recommendation of 75kg per year inhabitants (206g per day per capita) (Badmus and Yekini, 2011). In Nigeria, vegetable production has been ongoing for decades, providing employment and income for the increasing population, especially during the long dry season. Among the important indigenous vegetables, the fluted pumpkin seems to be widely eaten in Nigeria and cultivated for its edible succulent shoots and leaves as a backyard crop mainly by the Igbo tribe (Akoroda, 1990).

Telfairia occidentalis (Hook. F.) is a tropical vine grown in West Africa as a leaf

vegetable and for its edible seeds. Common names for the plant include Fluted gourd, Fluted pumpkin, and Ugu. *Telfairia occidentalis* is a member of the *curcurbitaceae* family. It originated from tropical West Africa (Irvine 1969; Esiaba, 1983) and is indigenous to southern Nigeria (Akoroda, 1990). The fluted gourd grows in many nations of West Africa but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines (Nwanna *et al*., 2008). Although the fruit is inedible, the seeds produced by the gourd are high in protein and fat, and can therefore contribute to a well-balanced diet. The plant is a drought-tolerant, perennial that is usually grown trellised. It is an herbaceous perennial crop although it is cultivated as an annual crop, especially under the traditional farming system in West Africa. The leaves are compound usually 3-5 leaflets, and the blades and petioles are covered with multicellular hairs which confer some measure of drought resistance on the plant. The fluted pumpkin is a fast-growing plant, the vines growing to 30.5m in length and it climbs by means of coiled tendril (Esiaba, 1982). It is a dicotyledonous plant. *T. occidentalis* is a dioecious climber, having a separate male (staminate) plant and female (pistillate) plant. *T. occidentalis* produces one of the largest-sized fruits among the cucurbits. It is also recalcitrant in nature (having poor seed storage and losing viability easily) (Akoroda, 1986). *T. occidentalis* is cultivated by seed and Southern part of Nigeria is the major area of production.

. Research on the genetic variability of the plant from different accessions will be used for a better understanding of crop evolution and improvement in the quality and quantity of seeds and leaves. In addition, morphological and molecular characterization of germplasm would aid plant breeders in selecting appropriate materials for further genetic improvement of cultivars.

2. MATERIALS AND METHOD

In Nigeria not much work has been done to access the genetic relationship in *T. occidentalis*, the bulk of the works of literature are based on the nutritional benefits and physiological characteristics, which are often influenced by the environment. Molecular biology tools are now employed for detecting variation in plants, by using polymerase chain reaction (PCR) which relies on the sequence information, to design and target specific primers (Welsh and McDeilland, 1990). There are several novel types of marker system that use PCR without prior information on the target DNA sequence. However, these sequences make use of a universal set of short oligonucleotides that amplify the DNA fragment randomly under low stringency conditions (Arif *et al.,* 2010). This strategy has enabled DNA polymorphism to be detected by primers amplification such as Random amplified polymorphic DNA (RAPD) analysis (Chan and Sun, 1997), arbitrarily; PCR analysis, DNA, amplification and fingerprinting analysis (Welsh and M.c. Deilland, 1990). RAPD Markers after the quick screening of different regions of the genome for genetic polymorphism have particularly been found to have intra-state application in plant molecular biology for the detection of genetic variation and construction of linkage maps in various plants and animals and in bulk segregate analysis for identifying markers linked to a gene of interest (Huang *et al*., 2003).

The aims and objectives of this study are;

1. To check if the collections of *T. occidentalis* from five different states are of the same line or different lines.
2. To check for inter-state and intra-state variation
3. To demonstrate the usefulness of RAPD markers in estimating the extent of genetic diversity in *T. occidentalis.*

Experimental Site: The research project was conducted at the experimental garden of the

Botany Department at Lagos State University Ojo. The vegetation on the plot is a mixture of *Imperata cylindrica* and has been on fallows for four years

Germplasm Collection: Twenty landraces or genotypes (pods) of matured fluted pumpkin fruits were obtained from the five states in South East and South West regions of Nigeria.

South East: – Anambra and Enugu States.

South West: – Ondo, Lagos and Ogun States.

Land Preparation: The experimental plot was cleared. The Zero tillage method was used.

Experimental Design: 20 accessions of *Telfaria occidentalis* were grown in the 2013 planting season. The experimental design used was a randomized block design. Pods from the five states were randomized within the main plots, while the genotypes from each state were randomized within the five subplots in each main plot. The main plot measures 7m x 10m and the subplots were single-row plants of 1m x 10m. Plot spacing was 1m each between and within rows. Alleys of 1m were created among replications.

Planting: After necessary data were collected on the pods and seeds, ten seeds were selected randomly from each pod and planted on the main and subplants allocated to each. **3.1.6 Cultural Practices:** Manual weeding was carried out at intervals of 2 weeks while fertilizer NPK 15:15:15 at the rate of 30g per plant was applied using the ring method of application to increase the fertility of the soil.

Data Collection: Ten morphological characters were collected on each genotype for agronomic yield and trait using appropriate equipment. The data collected are as follows:

a. Vine length two weeks after planting (cm). (from the base of the stem)
b. Number of leaves 2 weeks after planting (all open leaves on the plant)
c. Number of branches at harvest 6 weeks after planting (cm).
d. The total number of branches per vine was counted four weeks after planting.
e. Number of leaves 6 weeks after planting. The total number of leaves per vine was counted.
f. Vine length 6 weeks after planting (cm). The vine length from the crown or collar of the tip was measured with a meter rule 6 weeks after planting.
g. Petiole length 6 weeks after planting in (cm). The length of the petiole from point of attachment to the base of the leaf was measured with a transparent ruler four weeks after planting.
h. Leaf length 6 weeks after planting (cm). The length of the leaf, from the point of attachment to the vine, to the tip (apex) of the leaf blade was measured with transparent ruler, at four weeks after planting.
i. Internodes distance (cm). The length of the internodes from one node to another was measured with transparent ruler at six weeks after planting. Starting from the node.
j. The petiole length at harvest.

Statistical Analysis: Quantitative data were subjected to single linkage cluster analysis, using sequential and hierarchical and nested(SAHN) clustering option of numerical taxonomy system of Multivariate program(NTSYS) package, version 2.02j.

Molecular evaluation

Preparation of Reagents

1. **Extraction Buffer:-** A 250ml extraction buffer was prepared using 250ml ImTric Hcl^{at}PH8.0, 25ml 0.5m EDTA^{at}PH8.0, 50ml 5m Macl, 300ml sterile water, and 5g 1% polyvinyl pyrogalidone (PVP).
2. A soul volume of 20% sodium dodecyl sulphate (20% SDS) was prepared by adding 30ml of distilled water to 20g of SDS. This was stirred well to mix and dissolve, and the

resulting solution was made up to 50ml with distilled water.

3. Potassium Acetate (CH_3Cook): A 250ml of 5m potassium acetate was prepared by adding 100ml of distilled water, 123g of potassium acetate, and stirring well to mix and dissolve. The solution was made up to 250ml with distilled water.
4. Tris Acetic Acid (TAE Buffer): A 500ml volume of 0.5XTAE buffer was prepared by adding together in a conical flask. 5.4g of Tris base, 2.95g of Boric acid, 2ml of 0,5MTAE and 500ml of distilled water. This was autoclaved at 121^0C for 15 minutes.

   (EDTA) Ethylene ditetra acetic acid.

   A 250ml of 0.5m EDTA was prepared by adding 100ml of distilled water to 45.03g of ethylene ditetra amine acetic acid (EDTA) and mixing properly to dissolve. The pH was adjusted to 8.0

   Sodium chloride (NaCL)

   A 250ml volume of 5m NaCl was prepared by adding 150ml of distilled water to 5.6g of NaCl and stirring well to dissolve. The solution was then made up to 250ml with distilled water.

DNA Extraction

The DNA was extracted from the leaves using a modified protocol (Eppendorf tube centrifuge) based on procedures by Dellaporta *et al.* (1983) as outlined below;

1. 1.5g of fresh leaves of each of the samples were ground properly with mortar and pestle in 800ml of extraction buffer, preheated at 65^0C
2. The properly ground paste was transferred to a 15ml Eppendorf tube and 750ml of extraction buffer was added.
3. 50ml 20% SDS was added and mixed thoroughly with a vortex mixer. The tubes were allowed to incubate at 65^0C for 10mins.
4. 250ml of 5m potassium acetate was added again and a vortex with vortex mixer. The tubes were also allowed to incubate again at 0^0C for 20mins.
5. The tubes were allowed to spin at 20,000xg for 15mins in a centrifuge (13,000rpm in SS 24 rotator).
6. The supernatants were paired through miracloth and filtered into fresh tubes containing 500ml isopropanol.
7. This votexed again with a volex mixer and incubated at 20^0C in a deep freezer for 30mins.
8. The tubes were allowed to spin at 20,000xg for 15mins (13,000rpm in SS34 rotor).

   The supernatants were poured off and the tubes were allowed to drain, by inverting the tubes on a paper towel.
9. The pellets retained at the bottom of the tubes were redissolved in 350ml of 50mm Tris, 10mm EDTA, pH8, and transferred to Eppendorf tubes.

   These were spinned in the centrifuge for 10min to pellet insoluble debris.
10. The supernatants were transferred to fresh Eppendorf tubes and an equal volume of 1.1 phenol chloroform was added and vortexed. It was then spun in a centrifuge for 10min.

    Phenol chloroform takes care of the impurities in the cell wall.
11. The aqueous phase was transferred to fresh Eppendorf tubes and 1/10 volume 3m sodium acetate (75m.) and 500ml isopropanol were added.
12. This was properly mixed, followed by brief centrifugation in a centrifuge to pellet DNA.
13. The pellet was washed with 1ml of the cold 80% ethanol and the supernatant was discarded. The pellet was dried and dissolved in 100ml, 10mm Tris, 1mm EDTA at pH 7.0.

DNA Quantification

The concentration and purity of DNA isolated by the Dellaporta protocol outlined above were checked by the absorbance at 260mm and the ratio of absorbance at 260mm/280mm using a Nanodrop spectrometer.

PCR Amplification

The DNA samples were subjected to PCR amplification using four primers

These include

RAPD 1

OPRO 02

OPC 04

And 1 SCAR primer

The major work of the PCR machine includes

1. Denaturation
2. Annealing
3. Final extension

The reaction was carried out in a 20ml reaction tube consisting of 2ml of DNA and master mix, 0.5ml of PC mol, RAPD primers and the remaining 13.5ml was made of sterile deionised water.

The PCR amplification was carried out in an Eppendorf thermal cycler or PCR machine with an initial denaturation at 95^0C for 1min followed by 40 cycles of 95^0C for 30 seconds, 30 seconds annealing for 1minute.

This was followed by a final extension at 72^0C for 1 minute.

The PCR machine was allowed to keep the samples into fridge at the temp of 10^0C before electrophoresis

The PCR products were analyzed by gel electrophoresis.

The electrophoresis is to separate the DNA in order to check its molecular weight, using markers. The process is as follows:

1. 0.5g of Agerose powder was mixed with 10ml of TAE buffer in a conical flask and melted in a microwave oven.
2. The Agarose was then cooled to 55^0C – 60^0C and ethidum bromide was added before pouring into a gel tray prefixed with a comb. The content is allowed for 20 – 30 minutes to solidify.
3. The types at the end of the gel tray were then removed and the tray was placed in a ring with 0.5x TAE buffer (Trice Acetic acid) poured to cover the gel by at least 0.5cm.
4. The combs were removed before the samples were loaded into the wells. There was no dye used because the master mix used for PCR contained a green dye.
5. The samples were then run into the gel at a constant voltage (65V) for 1 – 2 hours until the dye migrated towards the opposite end of the gel.
6. After electrophoresis, the gel was viewed under ultraviolet light and photographed.

IKB DNA ladder was used as a DNA molecular weight standard to estimate the molecular weight of the amplified products.

For each decametre primer, a matrix of all the bands present in different DNA was generated when the band was present and when the band was absent.

RAPD 1	-	gcc aga tca g
OPRO 2	-	caca agc tgcc
OPC 04	-	ccg cat ctac
SCAR 1	-	tve gaa gcc ggt
		cat atc ga
		ve acc acg aat gtg tag

Single Linkage Cluster Analysis (SLCA)

Single Linkage Cluster Analysis reduced the number of genotypes into groups with the objective to minimize loss of information (Rhodes and Martins, 1972). Single linkage cluster analysis was performed to obtain a dendrogram and to sort genotypes into clusters based on the pattern of similarity and dissimilarity using the clustering procedure of SAS 2000. The procedure sorted the

genotypes into a dendrogram at intervals of 5% level of similarity starting from a minimum distance of 0.00 to 0.99 level of similarity when all the 20 genotypes occurred in a single cluster.
The fragment sizes of the PCR amplified products were estimated from the gel by comparison of the standard molecular weight of the marker.

4. RESULT:
Morphology: qualitative characters such as leaf and stem morphology did not significantly discriminate among the twenty landraces that were studied. For quantitative characters, vine length, leaf length, width, number of leaves, and internode distance showed huge variation for the twenty genotypes.

Morphological data analysis
The mean squares from analysis of variance (ANOVA) for the twelve agronomic traits of twenty fluted pumpkin genotypes were used to generate a UPGMA dendogram. . The procedure sorted the genotypes into a dendogram at intervals of 5% level of similarity starting from a minimum distance of 0.00 to 0.99 level of similarity when all the 20 genotypes occurred in a single cluster.

Molecular data analysis: The fragment sizes of the PCR amplified products were estimated from the gel by comparison of the standard molecular weight of the marker.
The four primers used produced polymorphic band patterns.
Opc – 04 gave 34 bands
Rapd I gave 100 band

The primers generated a total of 177 bands, in the size range of 250 – 1500Bp

Table 1: Means of twelve agronomic characters of twenty fluted pumpkin genotypes

Genotype	Characters											
	Days to emergence	Vine length 2WAP (cm)	Number of leaves 2WAP	Vine length 6WAP (cm)	Number of leaves 6WAP	Number of branches 6WAP	Vine diameter 6WAP (cm)	Leaf length 6WAP (cm)	Leaf width 6WAP (cm)	Leaf area 6WAP (cm^2)	Petiole length 6WAP (cm)	Internode length 6WAP (cm)
A1	7.67e	30.57bcd	7.00ef	71.90j	26.67d-g	1.67a	0.28b	12.08d	5.10de	153.95ef	4.37i	6.17i
A2	13.33a	31.63bc	8.67cd	128.47fg	31.00bcd	1.67a	0.48b	12.98c	6.10b	197.91c	5.10h	8.13f
A3	13.67a	32.83b	7.67def	154.33e	26.67d-g	0.67ab	0.46b	12.07d	5.27d	159.13e	6.93e	7.01g
A4	13.33a	29.97cd	10.33b	179.50d	30.67bcd	1.33ab	0.41b	15.23a	6.24ab	237.60a	6.67e	7.10g
E1	7.67e	16.17gh	6.33f	66.27j	22.33fg	0.00b	0.18b	10.23g	4.53f	116.04i	4.97h	4.08j
E2	8.67d	34.17a	7.67def	119.43g	27.33de	1.33ab	0.30b	11.20e	5.03de	140.95g	6.04c	9.87bc
E3	12.00b	32.23abc	8.33cde	147.67e	28.67cd	1.33ab	0.47b	11.18e	4.97de	138.88g	5.97f	6.23i
E4	12.00b	34.23a	7.67def	153.67e	34.33ab	1.67a	0.23b	11.22e	5.20d	145.93fg	7.71cd	6.03i
L1	9.67c	17.17fgh	8.67cd	120.68g	28.67cd	1.00ab	0.29b	13.17c	5.68c	187.10dd	6.23f	6.33hi
L2	10.00c	21.24e	10.67b	132.77f	27.67de	1.33ab	0.39b	11.14e	5.03de	140.20g	6.07f	9.27de
L3	7.67e	15.07h	6.33f	130.60fg	23.00efg	1.00ab	0.25b	10.17g	3.70h	94.09jk	7.43d	9.70cd
L4	8.00de	32.50ab	16.33a	237.23b	34.67ab	1.33ab	0.50b	15.07a	6.47a	243.58a	9.07b	10.30b
N1	10.00c	18.93ef	8.33cde	135.63f	31.33bcd	1.33ab	1.60a	10.07g	4.03g	101.50j	5.47g	6.97g
N2	9.67c	20.57e	9.67bc	149.00e	33.00abc	1.33ab	0.29b	10.63f	4.83ef	128.59h	6.13f	11.10a
N3	13.33a	17.57fg	6.67f	128.67fg	27.17dfg	0.00b	0.21b	6.90j	3.20i	55.15n	7.87c	6.70gh
N4	13.33a	28.83d	8.67cd	279.83a	36.67a	1.33ab	0.29b	9.17h	3.54h	81.13l	9.80a	9.33de
O1	7.67e	7.23i	7.67def	90.37i	22.00g	1.67a	0.23b	7.30i	2.85j	52.06n	3.97j	4.27j
O2	8.00de	17.10fgh	6.33f	146.73e	31.33bcd	1.33ab	0.31b	9.23h	2.90ij	66.88m	5.95f	6.77gh
O3	13.00a	31.09	10.33b	196.67c	29.00cd	1.33ab	0.41b	14.17b	5.97bc	211.31b	9.90a	8.91e
O4	12.00b	19.51ef	8.33cde	106.06h	27.67de	1.00ab	0.31b	9.30h	3.67h	85.29kl	5.91f	6.97g

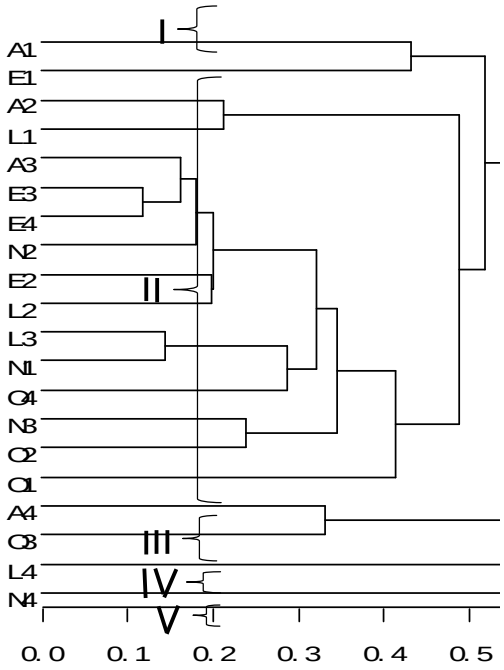
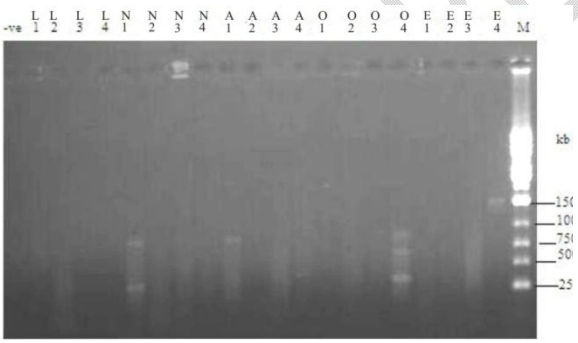


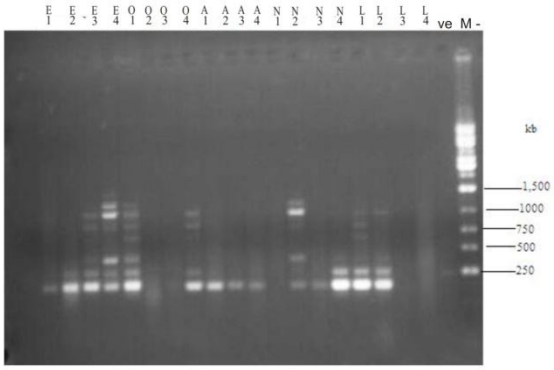
Fig. 1 Dendogram resulting from single linkage cluster analysis of twenty genotypes of fluted pumpkin.



OPC - 04

RAPD profile using primer OPC 04
The gell lane marked M, 1kb DNA ladder
The lane E1-E4 represent Enugu, 01-04 represent Ogun, A represent Anamabra, N1-N4 represent Ondo, L1-L4 repr Lagos.

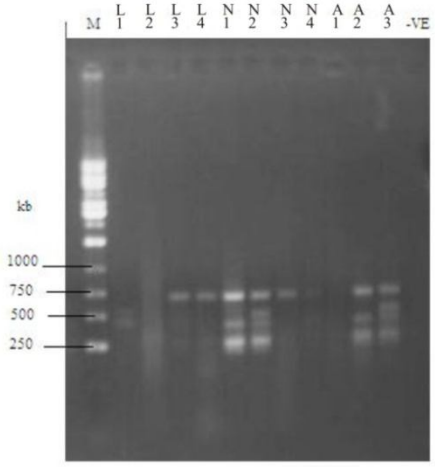
Fig. 2. RAPD profile using primer OPC-04



labelled RAPD -01

RAPD profile using primer radp - 01
The gell lane marked M, 1-5kb DNA ladder
The lane E1 - E4 represents Enugu, O1-O4 - Ogun, A1-A4 Anambra, N1-N4 Ondo, L1-L4 Lagos.

Fig. 3. RAPD profile using primer RAPD-01



labelled opr 02 (1-11)

RAPD profile using primer opr 02
The gell labe marked M, 1kb DNA ladder
L1-L4 represent Lagos, N1-N4 represent Ondo
A1-A4 represent Anambra, O1-O4 represent Ogun, E1-E4 represent Enugu

Fig. 4. Labelled opr 02 (1-11)

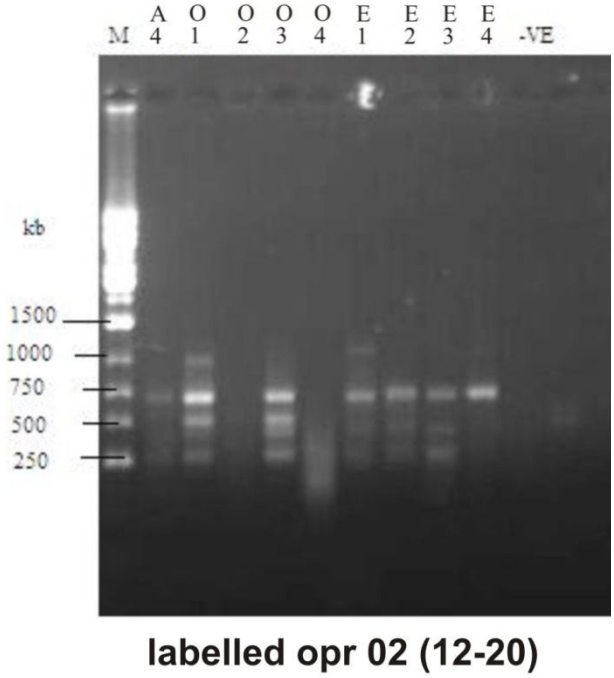


Fig. 5. Labelled opr 02 (12-20)

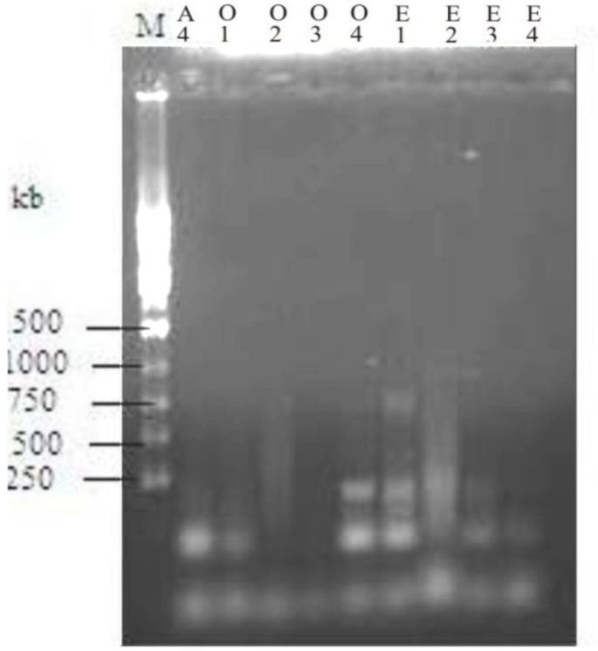
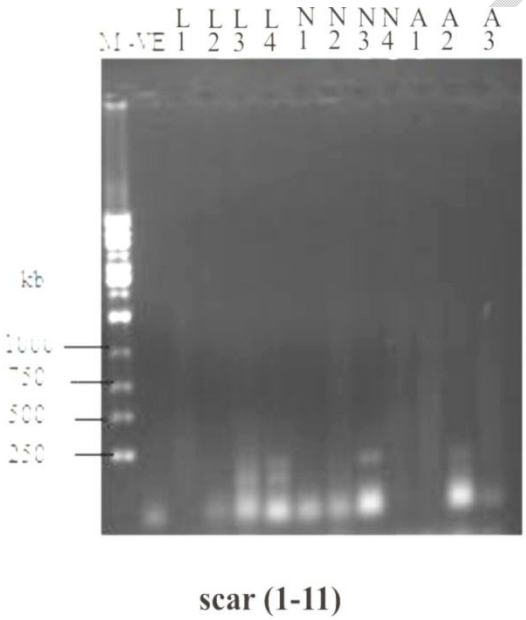


Fig 7. The RAPD profile of the plants



RAPD profile using SCAR primer

The gell lane marked M, with 1kb DNA ladder.

The Lane L1 - L4 represent Lagos N1 - N4 repres

A1 - A4 represent Anambra.

O1 - O4 represent Ogun, E1 - E4 represent Enugu

Fig. 6. RAPD profile using SCAR primer

The RAPD profile of the plants for the four primers is shown in figure above.

The four primers had a GTC content of 30 – 70% and the primer that generated the highest number of the band had a GTC content of 40% (RAPD I)

The DNA WAS confirmed by agarose gel electrophoresis

From the DNA picture, NO two primers had the same profile.

The genotypes showed variation in their band pattern indicating that diversity exists.

But genotypes A3 and N2 in primer opr -02 and E4 and N2 in rapd – 01 had equal bands and can be said to be clonally related despite they are from different states.

5. **DISCUSSION**

The existence of genetic variation can be employed as the basis for improving yield and other potentials of crop plants (Morakinyo and Makinde, 1991; Muhammad *et al*., 2007; Jonah

et al., 2010; Makinde and Ariyo, 2013). Therefore, knowledge of the genetic variability of growth characters would aid in the choice of an effective and efficient breeding method that will accelerate the pace of improvement in yield traits. In this study, the 20 genotypes of fluted pumpkin collected from 5 different states of Nigeria, showed wide ranges and significant variation The genotypes were grouped into five clusters by SLCA, each carrying fluted pumpkin genotypes sharing common properties and being similar to one another. There was no consistency in the clustering pattern of the genotypes. Some genotypes sourced from the same state were either grouped or not grouped together while others from different sources were clustered together. This has, in some cases been attributed to a lack of similarity between genetic and geographical diversity due to the movement of germplasm through seed exchange (Gwanama and Nichterien, 1995; Ntundu, 2002; Fayeun and Odiyi, 2012). According to Fayeun, 2011, the exchange of seeds is a common practice among African farmers. Odiaka *et al.* (2008) reported that commercial fluted pumpkin growers in the middle belt source their fluted pumpkin seeds from the South-Eastern states of Nigeria. Similar practices have been reported in Zambia for Zambian cucurbit (*Cucurbita moschata* Duch.) by Gwanama and Nichterien (1995), and in Tanzania for Bambara groundnut (Ntundu, 2002).

There was an obvious distinction in the mean values of characters across the clusters. Therefore, an improvement programmes in fluted pumpkins through the direct varietal selection might be easy (Fayeun and Odiyi, 2012). In addition, clusters that showed some character distinctions could be employed for hybridization purposes. Cluster I recorded the earliest days to emergence but poor in growth characters while cluster V was late in emergence but had the best performance in growth parameters, hence genotypes in cluster I may be selected for early emergence while cluster V may be selected for improved vegetative growth. In addition, a high-yielding progeny which will have a better combination of early emergence and improved vegetative growth could be selected from a cross between suitable entries in clusters I and V. A large amount of genetic variation observed among the genotypes supported the earlier observation by Odiaka (2005), Fayeun *et al*. (2012), and Odiyi *et al*. (2014) that abundant genetic divergence existed in fluted pumpkin germplasm. According to Makinde and Ariyo (2010), the pattern of genetic variation observed would be of great importance to germplasm collectors and plant breeders. The categorization of the diversity among the genotypes into groups with similar characteristics can be used to design a collection strategy (Ariyo, 1995; Makinde and Ariyo, 2010).

Molecular studies:

RAPD markers were also used to access the variation in the 20 genotypes from five states in Nigeria.

As Ogunkanmi et al (2010) opined, phenotypic markers are usually affected by environmental conditions, growth stages, and agronomic practices.

Chan and Sun (1997) states that morphological variation could cause confusion or misunderstanding and RAPD analysis is a powerful tool for determining genetic relationships.

Arif *et al.,* (2010) indicated that sequence-based analysis sometimes fails to distinguish between species because of the significant similarities between their DNA sequence in the amplified region.

They reported that RADP pimers distinguish below the specie level because RAPD analysis reflects both coding and non-coding regions of the genome.

Since each 2ml of oligoinucleotide primer used only covers a very limited part of the genome, important differences located on non-amplified regions could be missed.

When similar profiles (Equal band) are obtained from two different genotypes or samples using a particular primer, conclusions can be made that the two particular sample are the same. The RAPD data in this study showed that no two primers revealed identical profiles of the genotypes. E4 and N2 detected by the primer RAPD – 01 have equal bands and can be said to be identical or clonally related even though they are from different states. Also the profile of the genotypes A3 and N2 detected by the primer Opr – 02 are also similar identical. Showing or indicating that they are closely related and different from other genotypes.

6. SUMMARY AND CONCLUSION

This study has generated data on the intra species diversity among the specie of Telfaria occidentalis from five states in Nigeria using phenotypic and RAPD profiles.

From the results obtained in this study, conclusions can be drawn that

- RAPD profile of the four primers also showed diversity among the genotypes.
- Diversity among the genotypes is a result of the gene pool and the rest a result of environmental influence.

7. REFERENCES

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