academicJournals

Vol. 12(37), pp. 5567-5576, 11 September, 2013
DOI: 10.5897/AJB2013.11956
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genetic differentiation and inheritance of random amplified polymorphic DNA (RAPD) markers in pectoral spine phenotypic sub-groups of *Clarias gariepinus*

O. O. Oyebola[1]*, B. O. Omitoyin[1], A. E. Salako[2], E. K. Ajani[1] and M. O. Awodiran[3]

[1]Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria.
[2]Department of Animal Science, University of Ibadan, Nigeria.
[3]Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 23 August, 2013

Information on genetic relationship of phenotypically divergent sub-groups would be useful for better identification, utilization and management of species. Recent study revealed phenotypic divergence in a reservoir population of *Clarias gariepinus*. Genetic variability of polymerase chain reaction (PCR) products of the phenotypic divergent sub-groups was investigated in this study. Polymorphism and genetic variability were investigated in electrophoresed random amplified polymorphic DNA (RAPD)-PCR products of blood samples of twenty (20) *C. gariepinus* individuals. The population comprised of five (5) individuals of the non-peses phenotypic sub-group- individuals that did not possess anteriorly serrated pectoral spines denoted by S and fifteen (15) individuals of peses sub-groups that possessed anteriorly serrated pectoral spines denoted as C. Standard protocols were followed in analyzing six screened RAPD primers per individuals DNA fragment. Produced bands of pheno-grams were scored and analyzed to establish polymorphism as well as within and between sub-populations allelic variability using unweighted paired group method of algorithms (UPGMA) and dendrograms cluster analysis. Genotype data of individuals in the groups were tested for canonically significant discriminant grouping using discriminant function analysis (DFA). Results reveal that the primers were polymorphic: 746 bands were obtained from 63 detected loci which gave 80.95% polymorphism. Polymorphic information content (PIC) ranged between 0.18 and 0.49. Percentage polymorphic band were 78.00 and 69.84% for peses and non peses sub-groups, respectively. Dendrogram separated the population to two groups. All peses individuals were in one cluster while all the non-peses individuals were on the second cluster. Within group variations were also observed: DFA revealed that 100% of original phenotypically grouped cases were correctly classified. It was concluded that RAPD primers are suitable genetic markers for establishing variability in *C. gariepinus* sub-populations; the pectoral spine phenotypic groups are genetic variants and are potential varieties for the species. The results would have wide application in identification, utilization and management of genetic resources of *C. gariepinus*.

Key words: Random amplified polymorphic DNA (RAPD) marker, morphologic and genetic variability, *Clarias gariepinus*.

INTRODUCTION

Clarias gariepinus, introduced in several countries of Europe and Asia (Saad et al., 2009), has drawn the attention of aquaculturists because of its biological activities that include faster growth rate, resistance to

diseases and possibility of high stocking density. Species identification and its genetic structure is a crucial issue for the economically important species *C. gariepinus.* Phenotypic studies on specimens of this specie in Asejire dam (South-Western Nigeria) revealed presence of two phena groups that are identifiable by the presence and absence of anteriorly serrated (toothed) pectoral spines. Lather et al. (2010) reported that presence of genetic diversity as well as morphological characteristics in strains proposes a methodology for easy and quick isolation method for both research and industrial analysis. Hence, sustainable utilization of the phenotypic sub-groups would require information on genetic variability of the sub-populations.

Traditional methods like morphological, physiological and biochemical studies used for taxonomic identification are laborious and time consuming (Coulo et al., 1994). Application of molecular markers based on relative difference in deoxyribonucleic acid (DNA) sequences between individuals would detect more polymorphism than morphological and protein-based markers. However, genetic link between the subgroups has not been documented; therefore, we need to use a genetic approach to establish the genetic relationship between the morphologically dissimilar groups. Random amplified polymorphic deoxyribonucleic acid-polymerase chain reaction (RAPD-DNA) markers technique generates large number of loci, is less expensive and it requires no prior DNA sequence information to perform the assay (Christopher et al*.,* 2004).

Application of RAPD techniques has greatly increased the ability to understand the genetic relationships within species at the molecular level (Sabir et al*.*, 2012). It was used in establishing clear image about phylogeny and genetic relations between local adaptive breeds in an attempt to generate information for future genetic improvement (El-Rabey and Al-Malki, 2011) and it is a tool for generating taxon-specific markers with different specificities (Day et al., 1997). This technique has been used to assess the genetic variability in animals such as buffalo, cattle, goat, and sheep (Appa Rao et al*.,* 1996), fish (Bardakci and Skibinski, 1994), bacteria (EL Hanafy et al*.,* 2007) and date palm (Soliman et al., 2003). This study utilized RAPD markers in assessing genetic variability of the phenotypically divergent *C. gariepinus* sub-populations.

MATERIALS AND METHODS

Sample collection, DNA extraction and RAPD amplification

Twenty (20) live samples of *C. gariepinus* were used for this study. The samples were randomly selected from a collection of the species obtained from Asejire dam (South Western, Nigeria) between December, 2009-November, 2011. The samples were obtained from catches of a bi-monthly sampling of set gura trap combined with fishermen's landings at the study site. *C. gariepinus* was identified using dorsal and anal fin ray counts following identification key (Teugels, 1986). The samples were re-grouped to two (2) classes based on their phenotypic characteristics. Individuals possessing the anterior serration on their pectoral spines were referred to as peses and were denoted by C while those without the anterior serration were referred to as smooth/non-peses and were denoted by S. Number of individuals selected per group was determined based on their relative proportion in the obtained population. Information on the RAPD DNA analysis is presented in Table 1. Fish identification, phenotypic characterization/sub-grouping and blood collection were carried out at the Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria. About 2 ml of blood was drawn from vertebral column of individual fish with the aid of hypodermal needle. The drawn blood were released into heparinized sample bottles and transported inside iced container to the Federal University of Agriculture Abeokuta, Nigeria (FUNAAB) where DNA extraction was carried out.

DNA extraction and dilution

Blood genomic DNA was extracted from the studied individuals using Norgens Blood Genomic DNA Isolation Kit (NORGEN, Biotec. Corporation). DNA extraction and purification followed the protocols of Hillis et al. (1996). Quality of DNA was checked by spectrophotometry taking ratio of optical density value at 260-280 nm. 1:100 DNA dilutions was obtained for 10 ul of each extracted DNA.

PCR mix preparation and gel run

RAPD-PCR product was prepared at the International Institute for Tropical Agriculture (I.I.T.A), Ibadan, Nigeria. PCR-mix contained: 10X Buffer (2.0 µl), 25 mM $MgCl_2$ (1.6 µl), 5%Tween 20 (2.0 µl), 2.5 mM dNTPs (1.0 µl), 2.0 mM primer (1.0 µl), 5µ/µl Taq (0.2 µl), water (8.2 µl), diluted DNA (4.0 µl). The PCR mix for each sample was spin down at 10,000 rpm for 30 s inside eppendorf 5415C. Amplification of PCR mix involved denaturation, annealing and extension processes. Thermal cycler (Techne, TC412) was utilized for amplification. The thermal cycle profile comprised of 1 cycle of 3 min initial denaturation at 94°C, 20 s at 94°C, 45 cycles of 20 s at 94°C, 20 s annealing at 37°C, 40 s at 72°C, and 1 cycle of 7 min final extension at 72°C. PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide done under standard electrophoresis procedure. Six (6) random amplified polymorphic DNA primers (OPERON primers) used for this study was obtained from Operon Technologies Inc. Alameda, California, EUA. The primers identities are presented in Table 2. Gel products were photographed and subsequently analysed for polymorphism.

Determination of polymorphic primers

A set of 20 decamer RAPD primers were initially screened before selecting some of them for this study. Primers screening was carried using three randomly selected samples as template. Presence of polymorphism and clarity of resolution was used in

*Corresponding author. E-mail: olusegun.oyebola@yahoo.com.

Abbreviations: RAPD-DNA, Random amplified polymorphic deoxyribonucleic acid-polymerase chain reaction; **PIC,** polymorphic information content; **RMS,** rotational mating selection.

Table 1. Information on the RAPD-DNA analyzed individuals.

Sample number	Score	Group
1	1	C
2	1	C
3	1	C
4	1	C
5	1	C
6	1	C
7	1	C
8	1	C
9	1	C
10	1	C
11	1	C
12	1	C
13	0	S
14	1	C
15	0	S
16	1	C
17	0	S
18	0	S
19	0	S
20	1	C

Group C individuals had complete anteriorly serrated pectoral spine and their score =1 while group S individuals had smooth anteriorly pectoral spine and their score = 0.

selecting the best 6 primers which were subsequently used for RAPD analysis of the 20 individuals.

Band scoring and data analysis

RAPD gel profile of each primer was scored across electrophoretic lanes as variables. Data were recorded as present (1) and absent (0) of band products from the gel electro-phenographs. Polymorphic data analysis followed Lathar et al. (2010). The generated binary data were used to estimate polymorphism level by dividing the polymorphic bands by the total number of scored bands. Polymorphic information content (PIC) was calculated by the formula: PIC = 2 Pi (1-Pi), where Pi is the frequency of occurrence of polymorphic bands in different primers. Amplified alleles were labeled alphabetically and frequencies of alleles determined. Presence of private allele in the groups and individuals were observed. Establishing genetic differences from the generated data and dendrogram drawn followed the methods of Saad et al. (2009).

Degree of genetic similarity, interrelationship among the studied individuals and calculation of similarity values were carried out using SPSS 15.0- Windows Evaluation Computer Package. The data were analyzed according to binary values 0 and 1. Where, 0=band absence; 1=band present. Results showed both hierarchical pair wise distance using unweighted paired group method of algorithms (UPGMA) and constructed dendrogram. Similarity was observed between all primers and between individuals genotypes with dendrogram constructed in both cases. Genotype data of individuals in the groups were tested for canonically significant discriminant grouping using discriminant function analysis (SPSS, version 15.0 computer software).

RESULTS AND DISCUSSION

The population analyzed in the study comprised 5 individuals of the non-peses group (13, 15, 17, 18 and 19) while the rest were in peses sub-group. Table 2 which showed the Polymorphic RAPD primers in *C. gariepinus*: their code, sequence and size range also revealed that the primers were within 150 and 3500 base pairs. Characteristics of the selected primers with respect to the studied population are presented in Plates 1 to 6 while result of the phenograms analysis for polymorphism is shown in Table 3. The result shows that RAPD primers were polymorphic and were able to detect private allele in the studied population. A total of 746 individual bands were obtained from a total of 63 detected loci which gives 80.95% polymorphism. However, the highest number of amplified fragments (13) was produced by OPAF-07. Number of polymorphic bands per primer ranged between 7(OPAE-04 and OPAE-05) and 11(OPAF-07). PIC ranged between 0.18(OPAF-08) and 0.49 (OPAE-05). Dendrogram constructed from the scored bands of the primers presented in Figure 1 shows that they clustered into two groups with intra and inter group variations. Primers OPAD-09, OPAE-04 and OPAF-08 clustered and were differentiated from the rest of the three. Table 4 shows information on occurrence of private allele by which the pectoral spine sub-groups of *C. gariepinus* can be differentiated. Despite similar values of percentage polymorphic band, private alleles were encountered in individuals of both subgroups. However, bands were more polymorphic in the peses group than the non peses (78.00 and 69.84%PB, respectively). Specific homogeneous sites were obtained in 11 cases. All individuals in both groups inherited allele j and k in OPAE-09. However, all loci were heterogeneous in OPAD-09 in peses group while two were homogenous in non peses group. Homogeneity of a particular allele in all members of a group could indicate its suitability as a marker for such group. OPAF-07 was differentially inherited by the two groups; it was uniformly inherited at one site (i) by all individuals in the peses group only. This makes it a potential differentiating site for the phena group. Also, OPAD-09 showed no private allele in peses indicating that the marker is not informative for the category peses but had 2 private alleles in the non-peses thus showing a sub-division or variant in this category. The genetic analysis confirmed the morphological assignation of each *C. gariepinus* groups based on pectoral spine but also highlighted subtle genetic intra-variability. The later being able to give further information on genetic basis of morphologically divergence groups and was able to show within sub-group genetic variability pattern. Dendrogram showing the cluster analysis of the individuals' genotype is presented in Figure 2. The UPGMA cluster diagram identified two major genotypic

Table 2. Polymorphic rapd operon primers in *c. gariepinus*: their code, sequence and size range.

S/N	Primer code	Sequence (forward)	Size range (bp)
1	OPAD - 09	TCGCTTCTCC	200 - 3500
2	OPAE - 04	CCAGCACTTC	250 - 2500
3	OPAE - 05	CCTGTCAGTG	150 - 3000
4	OPAE - 09	TGCCACGAGG	200 - 3000
5	OPAF - 07	GGAAAGCGTC	250 - 3000
6	OPAF - 08	CTCTGCCTGA	150 - 3500

bp, Base pair.

Table 3. Primer code, total number of band locus detected (NBL), number of polymorphic band (NPB), average polymorphic band (%PB), polymorphic information content (PIC), private allele per primer (NO. of PA), total number of individual band per primer (NIB) and relative band frequency (Av.BF) generated by the six RAPD primers.

Primer code	NBL	NPB	%PB	PIC	No. of PA	NIB	Av.BF
OPAD - 09	9	8	89.00	0.20	0	82	0.11
OPAE-04	9	7	77.78	0.3457	0	102	0.14
OPAE-09	11	9	81.82	0.2975	0	137	0.18
OPAF-08	10	9	90	0.18	0	104	0.14
OPAE-05	11	7	54.55	0.4959	0	137	0.18
OPAF-07	13	11	76.92	0.3551	1(14)	184	0.25
Total	63	51			1	746	

NBL, Number of band locus; NPB, number of polymorphic band; %PB, percentage polymorphic band; PIC, polymorphic information content; NIB, number of individual band.

M 1 2 3 4 5 6 7 8 9 10 1112 1314 1516 17 18 19 20

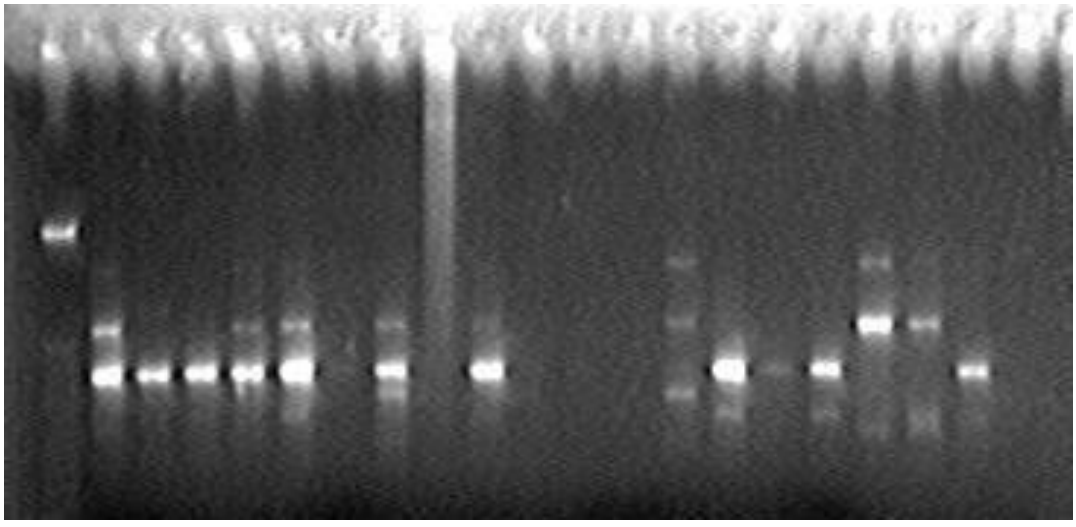

Plate 1. Gel phenogram of OPAD - 09

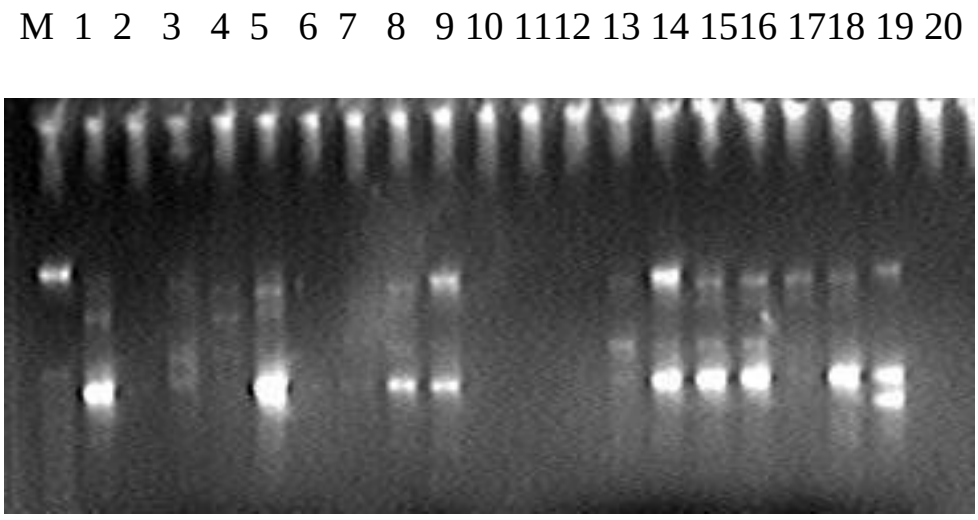

Plate 2. Gel phenogram of OPAE 04.

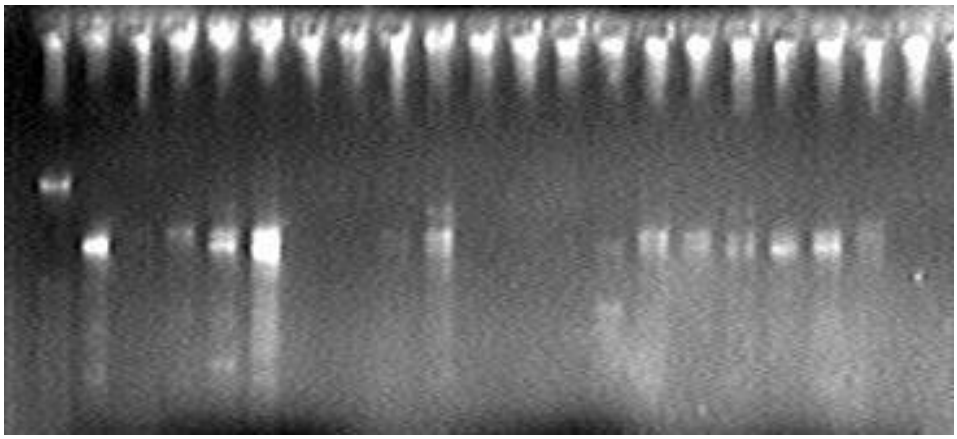

Plate 3. Gel phenogram of OPAE 09.

groups with inter and intra group relationships. It also confirmed genetic background for phenotypic separation of the population via pectoral spine; all individuals in the first cluster were from the peses group while all the non peses individuals were on the second cluster. However, all the groups had varied interrelationships showing a highly heterogeneous population. Classification statistics (Table 5) revealed that the initial phenotypic grouping was 100% which is in accodance with the genotypic grouping obtained in this study.

The study reveals that RAPD markers were suitable in establishing polymorphism in the sub-groups of *C. gariepinus*. Genetic disparity between *C. gariepinus* and some other catfishes using molecular tools has been reported by Galbusera et al. (1996), Agnese and Teugels (2001) and Na-Nakorn et al. (2004). Application of the RAPD technique in several fish characterization and genetic variation studies has been reported in Bardakci (2001). The result of the current study indicates that the RAPD-PCR analysis is equally suitable in establishing genetic variability in sub-populations of *C. gariepinus*. The RAPD primers were polymorphic in the population and its sub groups, it also established pattern of intra and inter group variations thus showing the efficiency of the RAPD primer in molecular genetics studies in the populations. It also supported the usefulness of the RAPD primer in genetic studies in *C. gariepinus* as discussed by Saad et al. (2009) and in genetic variability

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 1516 1718 19 20

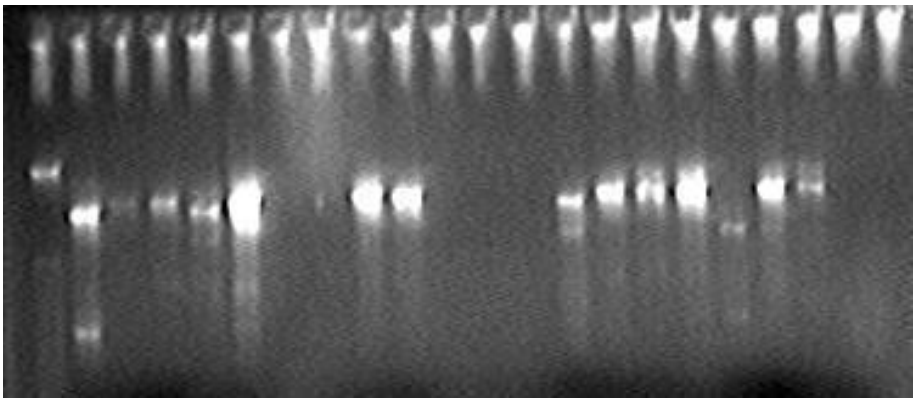

Plate 4. Gel phenogram of OPAF -08.

M 1 2 3 4 5 6 7 8 9 10 1112 1314 15 161718 19 20

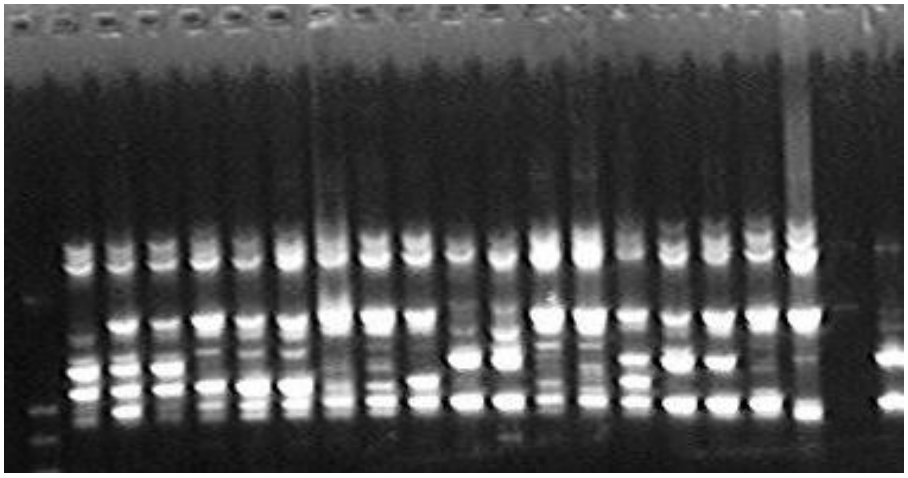

Plate 5. Gel phenogram of OPAE-05.

studies as reported in Almeida and Sodre (2002), Quibai et al. (2006) and Hung et al, (2005).

Knowledge on genetic variation in *genus Clarias* is important as it would facilitate better identification (Teugels et al., 1992; Agnese et al., 1997;, Rognon et al., 1998) as well as assist in detection of introgression and hybridization with other species (Billington et al., 1996). Result of the investigation on genetic variability of the studied population revealed variation in both within and between sub-groups of *C. gariepinus*. This indicates that the population expressed genetic heterogeneity. The result thus agreed with the earlier reported observation of heterogeneous phenotypic structure of the population. However, genetic variability of the stock will have to be maintained in order to sustain the genetic potential of the fishery. This is because of the reported continual decreasing population size of *C. gariepinus* in Asejire dam coupled with expanding pressure on its use for research and mass propagation. Smallness of population in fragmented catchment like the study area will facilitate in-breeding and its attendant depression in the future. However, the variability pattern as documented in this study would be useful in monitoring and maintenance of *C. gariepinus* genetic pool in the catchment. Maintenance of stocks genetic variability would involve minimizing mating of closely related individuals.

Saad et al. (2009) reported that failure to maintain stocks genetic variability in *Oreochromis niloticus* families

M 1 2 3 4 5 6 7 8 9 10 1112 13 14 1516 1718 19 20

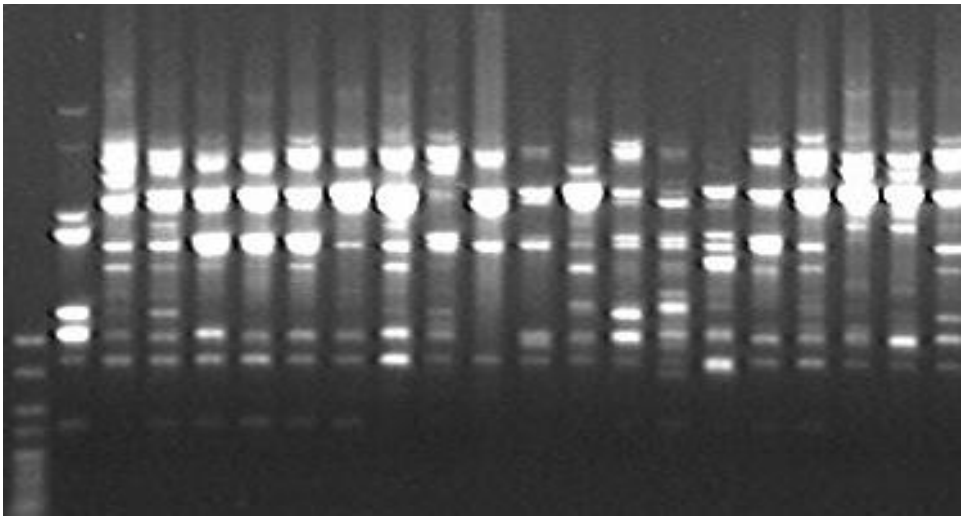

Plate 6. Gel phenogram of OPAF-07.

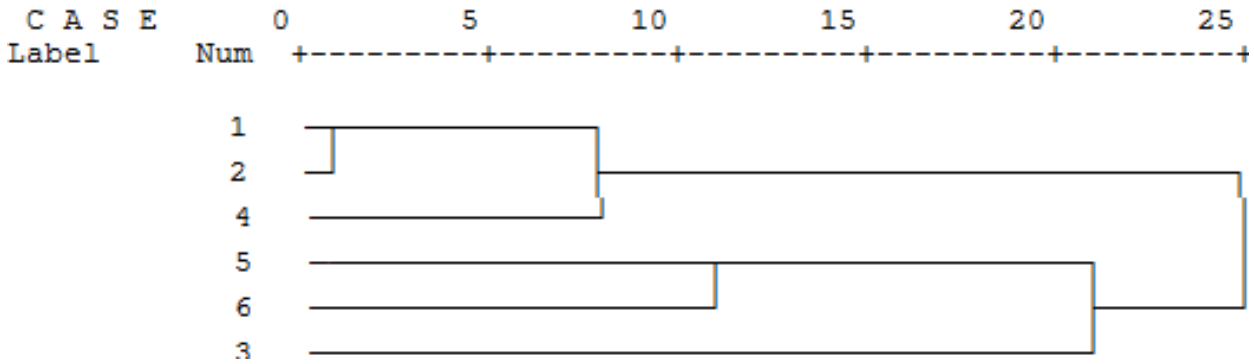

Figure 1. Dendrogram showing average linkage between the six polymorphic primers with respect to the studied population (primers clustered into two groups with intra and inter group variations). 1, OPAD-09; 2, OPAE-04; 3, OPAE-09; 4, OPAF-08; 5, OPAE-05; 6, OPAF-07.

Table 4. Occurrence of private allele by pectoral spine sub-groups of *Clarias gariepinus* after RAPD primers analysis.

Primer code allele	No. of Homogeneous sites		
	Peses	Non-peses	Differentiating
OPAD-09	0	2 (b,l)	2(b,i)
OPAE-04	2(h,i)	4 (e,g,h,l)	2(e,g)
OPAE-09	2(j,k)	2 (j,k)	-
OPAF-08	1(j)	3(e,g,j)	2(e,g)
OPAE-05	6(c,d,g,h,l,j)	6(a,b,c,d,h,i)	4(a,b,g,i)
OPAF-07	3(c,d,i)	2(c,d)	1(j)
Total (MB)	14	19	11
%PB	78	69.84	

*MB, Monomorphic band; PB, polymorphic band.

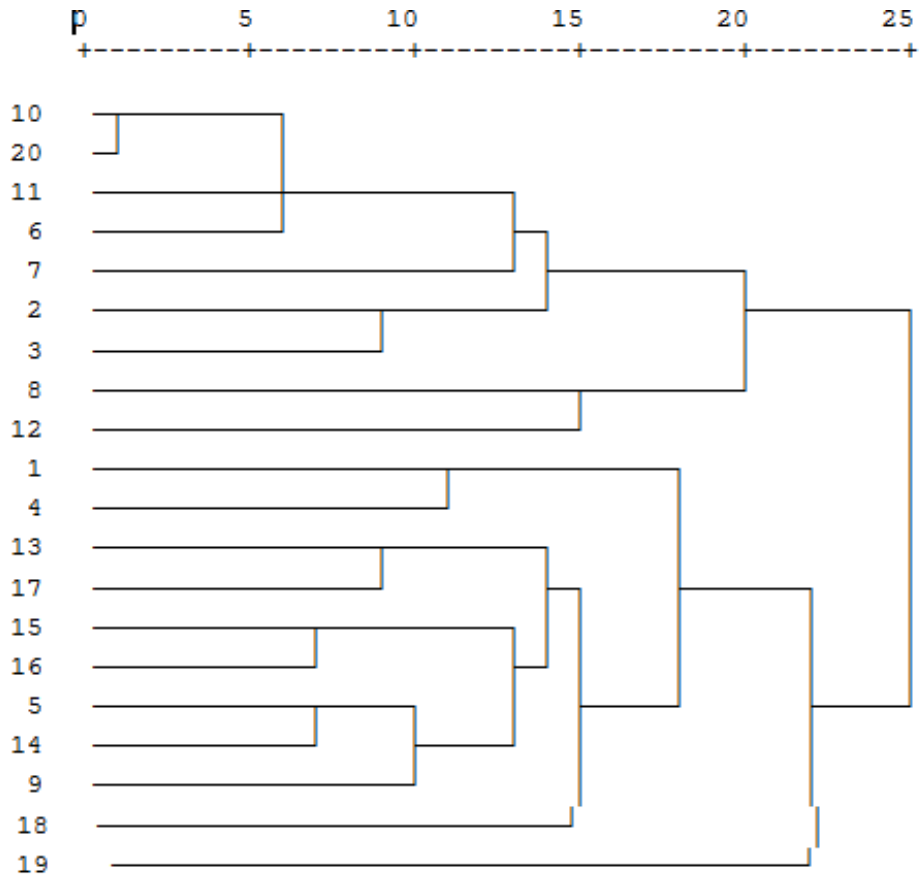

Figure 2. Dendrogram representing the inferred phylogenetic relationship in *C.gariepinus* population based on RAPD analysis. Individuals 13, 15, 17, 18, and 19 belong to S group while others belong to C group.

Table 5. Classification results (a) for the C. *gariepinus* population's genotypes.

Parameter	Score	Predicted group membership		Total
		0.00	1.00	0.00
Original count	0.00	5	0	5
	1.00	0	15	15
%	0.00	100.0	0.0	100.0
	1.00	0.0	100.0	100.0

a = 100.0% of original grouped cases correctly classified.

could be attributed to un-minimized mating of closely related individuals. However, minimizing mating of closely related individuals in the study area may not be feasible going by its observed fragmented structure as observed in an earlier assessment of the catchment and the declining state of *C. gariepinus* stock in the catchment (Omoike, 2004; Oyebola et al. *in press*). Moreover, this will be heightened by the pressure on its fishery as major source of wild brood-stock for research and mass propagation (FAO, 2012), in hatchery stock improvement coupled with its reproductive versatility (Nukwan et al., 1990) and the sporadic growth of its hatcheries in the region. However, collections from the capture environment could be isolated in special hatchery under breeding programmes and produced through rotational mating selection (RMS) method (PNGS, 2007) but this has to be done under restricted management in order to achieve the desired objective (Saad et al., 2009).

Canonical classification analysis of the genotypic data showed 100% differentiation of the sub-groups genotypes, presence of private alleles and the sub-groups can be differentiated using OPAF-07. These indicate a potential advantage in marker assisted selection for these potential *C. gariepinus* varieties. This observation may have implications apart from taxonomy. Saad et al. (2009) reported that generated RAPD-DNA markers may be associated with DNA regions which affect economic characters. Moreover, earlier study on biochemical differentiation of the sub-groups had revealed a differentiating marker that has nutritional and medical importance. The identified locus in the current study may therefore be confirming that the earlier observations has DNA basis and their differences would be heritable. The UPGMA dendrogram agreed with the phena classification of the pectoral spine groups thus indicating that the sub-groups are genetically different. Within population variation was observed in both groups which indicated that the populations were genetically heterogeneous. Earlier phenotypic studies on the population had revealed that *C. gariepinus* population obtained from the Asejire dam was heterogeneous and within sub-groups variation existed. The current result may therefore be confirming that the pattern has genetic basis.

In conclusion, the pectoral spine variants are genetic variants and are potential varieties for *C. gariepinus* while the RAPD primers were suitable genetic markers for establishing variability in the populations. Bowditch et al. (1993) reported that detection of genetic variation is essential to a wide range of comparative genetic research endeavours which include gene mapping, individual identification, parentage determination, population genetics and molecular phylogenetics. The obtained result in this study would therefore have wide application in utilization and management of genetic resources in *C. gariepinus*.

ACKNOWLEDGEMENTS

The efforts of Mr. Alonge of the Bioscience Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and Mr. Oyelakin of the Central Biotechnology Laboratory, Federal University of Agriculture, Abeokuta, (FUNAAB), Nigeria are well appreciated for their technical assistance.

REFERENCES

Agnese JF, Teugel GG, Galbusera P, Guyomard R, Volckaert F (1997). Morphometric and Genetic Characterization of Sympatric Populations of *Clarias gariepinus* and *C. anguillaris* from Senegal. J. Fish Biol. 50:1143-1157.

Agnese JF, Teugels GG (2001). Genetic Evidence for Monophyly of the Genus *Heterobranchus* and Paraphyly of the Genus *Clarias* (Siluriformes, Clariidae).Copeia 2001(2):548-552.

Almeida FS, Sodre LMK (2002). Comparative Study by RAPD analysis of six species of the *Pimelodidae* family (*Osteichthyes, Siluriformes*) from the Tibagi River, State of Panama, Brazil. Maringa 24:513-517.

Appa Rao KBC, Bhat KV, Totey SM (1996). Detection of species specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD) genetic analysis. Biomol. Eng. 13:135-138.

Bardakci F (2001). Random Amplified Polymorphic DNA (RAPD) Markers. Turk. J. Biol. 25:185-196.

Bardakci F, Skibinski DOF (1994). Application of the RAPD technique in Tilapia fish species and sub-species identification. Heredity 73:117-123.

Billington N, Brooks RC, Heidinger RC (1996). Use of cellulose acetate electrophoresis to rapidly screen sauger broodstock for sauger-walleye hybrids. The Progressive Fish-Culturist 58:248-252.

Bowditch BM, Albright DG, Braun MJ, Michael JB (1993). Use of Randomly Amplified Polymorphic DNA Markers in Comparative Genome Studies. Method. Enzymol. 224:294-309.

Christopher W, Theodorakis I, John W (2004). Molecular markers. Ecotoxicology 13:303-309.

Coulo MMB, Vessen JMBM, Hofstra H, Huisveld JHJ (1994). RAPD analysis: a rapid technique for differentiation of spoilage yeasts. International. J. Food Microbiol. 24(1):249-260.

Day WA Jr, Pepper IL, Joens LA (1997). Use of an arbitrarily primed PCR product in the development of a *Campylobacter jejuni* -specific PCR. Appl. Environ. Microbiol. 63:1019-1023.

El-Hanafy AA, Abd-Elsalam HE, Hafez EE (2007):Fingerprinting for the lignin degrading Bacteria from the soil. J. Appl. Sci. Res. 3:470-475.

El-Rabey H, AL-Malki A (2011). Application of Randomly Amplified Polymorphic DNA (RAPD) Markers and Polyphenol Oxidases (PPO) genes for distinguishing between the diploid (Glaucum) and the tetraploid (Leporium) accessions in *Hordeum murinum* complex. Afr. J. Biotechnol. 10 (61):13064-13070.

FAO (2012). Cultured Aquatic Species Information Programme; *Clarias gariepinus* (Burchell 1822) www.fao.org.

Galbusera P, Volckeart FA, Hellemanns B, Ollevier F (1996). Isolation and Characterization of Microsatellite Markers in the African Catfish *Clarias gariepinus* (Burchell 1822). Mol. Ecol. 5:703-705.

Hillis DM, Mable BK, Larson A, Davis K, Zimmer E (1996). Nucleic acids IV:Sequence and cloning. In Hillis, D.M.,Moritz, C. and Mable, B. (Eds.), Molecular systematic 2nd Eds., 342-343 Sunderland, Massachusetts Sinauer Associates, Inc.

Hung C, Hsuan LY, Chen DJ (2005). The use of RAPD markers to assess Catfish hybridization. Biodivers. Conserv. 14:3003-3014.

Lathar PK, Sharma A, Tharkur I (2010). Isolation and Random Amplified Polymorphic DNA (RAPD) analysis of Wild Yeast Species from 17 different fruits. J. Yeast Fungal Res. 1(8):146-151.

Na-Nakorn U, Kamurat W, Ngmsiri T (2004). Genetic diversity of Walking Catfish, *Clarias macrocephalus*, in Thailand and Evidence of Genetic Introgression from Introduced Farmed *Clarias gariepinus*. Aquaculture 240:145-163.

Nukwan S, Lawanyawut K, Tangtrongpiros M, Veerasidth P (1990). Backcrossing experiment of hybrid between *Clarias macrocephalus* and *Clarias gariepinus.* Proceedings of the 28th Kasetsart University Conferences, Kasetsart University, Bangkok. pp. 529-544.

Omoike A (2004). Sustainable Management of Fisheries of Asejire Reservoir and its Environs in Southwestern Nigeria. Ph.D. thesis, University of Ibadan, Ibadan, Nigeria. p. 186.

PNGS (2007). Producing pure lines of the Egyptian Nile tilapia using molecular genetic techniques and selection. Report of Programme of National Strategy for Biotechnology and Genetic Engineering, Science and Technology Center, Academy of Scientific Research and Technology, Ministry of Scientific Research, Egypt.

Quibai Z, Fengbo I, Li Z, Jianfang G (2006). RAPD markers between Yellow Catfish (*Pelteobagrus fulvidraco*) and long whiskers Yellow Catfish (*P. enpogen),* Acta-Hydrobiologica-Sinica 30:482-485.

Rognon X, Teugels GG, Guyomard R, Galbusera P, Andriamanga M, Volckaert F, Agnese JF (1998). Morphometric and allozyme variations in the African catfishes *Clarias gariepinus* and *C. anguillaris*. J. Fish Biol. 53:192-207.

Saad YM, Ali SF, Hanafi MS, Ezza MA, Guerges AA (2009):Genetic Signature of Some Egyptian *Clarias gariepinus* Populations. Global Veterinaria 3(6):503-508.

Sabir JSM, Mohamed HZM, Amr A El-Hanafy, Mohamed MA (2012). Genetic similarity among four breeds of goat in Saudi Arabia detected

by random amplified polymorphic DNA marker. Afr. J. Biotechnol. 11(17):3958-3963.
Soliman SS, Ali BA, Ahmed MMM (2003). Genetic comparisons of Egyptian date palm cultivar (*Phoenix dactylifera L*) by RAPD-PCR. Afr. J. Biotechnol. 2:86-87.
Teugels GG (1986). Morphology Data of *Clarias gariepinus*; Identification Keys. http://fishbase.sinica.edu.tw/physiology/MorphDataSummary.
Teugels GG, Guyomard R, Legendre M (1992). Enzymatic variation in African clariid catfishes. J. Fish Biol. 40:87-96.