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Genetic and Biochemical Basis of Growth Variation in Bred *Clarias gariepinus* (Burchell 1822)

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Challenges of size variants (shooters and runts) in bred *Clarias gariepinus* could be solved if relevant information on the basis of the heterogeneity is known. Genetic and biochemical basis of growth variation in *C. gariepinus* was investigated in this study. Tissue samples of four fastest growers- S (4.16±2.40g)) and least four growers- R (0.59±0.83g) in a 6weeks old population of bred *Clarias gariepinus* were subjected to Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Bands were analyzed for differentiating locus for S and R. These loci were compared with kDa ladder to determine molecular weight and biochemical implications. Variants' genotypes were analyzed for phylo-genetic relationship using cluster analysis. At <14.7 and >150kDa loci, S and R were differentiated. These loci correspond with α-amylase inhibitor and polymorphic Prostatic Growth Factor-1 (PGF-1) locus respectively. At 0.75 coefficient of similarity, genotypes grouped to two major clusters. All R-group members and one of S-group members were on a cluster while all other S-group members formed another cluster. In the studied C. *gariepinus* population, growth heterogeneity has genetic and biochemical basis. Fast growers were more genetically versatile. Obtained knowledge is important in discrimination and utilization of fast growers, Marker Assisted Selection (MAS) and breeding programmes planning.

Keywords: Growth variation, genotype, Biochemical differentiation, phylogeny, *Clarias gariepinus*

INTRODUCTION

Nigeria is the largest producer of the mud catfish (*Clarias gariepinus*) in sub-Sahara Africa, and the last twenty years has seen considerable gains in knowledge concerning its reproduction and rearing (Megbowon et al., 2013). The *Clarias gariepinus* is a species of high aquaculture importance (Odedoyin, 2007). It is widely cultured owing to its high market price, fast growth and ability to withstand adverse pond conditions. These attributes has encouraged more farmers to show interest in its seed production. However, size variation in Clariids spawned at the same time from the same or mixed brood-stock is an outstanding problem in catfish farming especially in Nigeria. This is because growth heterogeneity has been associated with cannibalism and therefore with mortality (Baras and d'Almedia, 2001; Baras and Jobling, 2002). Cannibalism is one of the several constraints in maximizing the production and survival of fish (Appelbaum and Arockiaraj, 2010).

Considerable growth (weight) variation occurs in *C. gariepinus* just like in most of the fish species reared in aquaculture. Field experience showed that although fast, medium and slow growers are normally encountered; the fast growers are considered as major challenge in maximizing production. In most cases, fast growers are of inadequate number for stocking, highly competitive and cannibalistic. The fast-growing cannibals may continuously prey on slow-growing conspecifics due to growth advantage through cannibalism (Ribeiro and Qin, 2015). In biological organisms, growth variation could occur due to biological and cultural factors (Stinson, 2012). Scientists have sought to present reasons and solutions to size variation: Madu and Ibikunle (1989) reported that size variation persisted among fry hatched from uniformly sized eggs. However, they observed that there was differential hatched time among the uniformly sized eggs. Uka et al. (2005) observed that hybridization of *Clarias gariepinus* with *Heterobranchus bidorsalis* couldn't eradicate size variation in these Clariids while Ayinla and Nwadukwe, (1990) were of the opinion that the proportion of fast growing fingerlings (shooters) increased according to the size of breeders; although, use of predominantly large breeders could not abolish the occurrence of size variation in *Clarias gariepinus* offspring.

Social hierarchies have been identified as the most important factor that is responsible for the growth variation in group-housed fish (Metcalfe et al, 1995 and Metcalfe, 1998). Management of fast growers has focused on this perspective. According to Biu et al. (2015), small fish are consumed by the larger ones and this cannibalism could be controlled by frequent sorting of the fast growers. Hence, fast growers are usually graded out of stocks and discarded in order to maximize production in commercial fish hatcheries. This is usually done with the expectation that grading minimizes the stress imposed by the larger (supposedly dominant) over small (supposedly subordinates) individuals. This practice result in improved growth of small individuals and increased total biomass output (Gunnes, 1976; Popper et al, 1992; Brzeki and Doyle, 1995; Seppä et al, 1999).

However, the management technique has given differential outcome: Biu, et al. (2015) reported better fish fingerling yield when shooters were removed frequently. Small sized fish were reported to compensate their growth after grading (Gunnes, 1976; Popper et al, 1992; Brzeski and Doyle, 1995; Seppä et al, 1999) whereas size grading did not had effect in Arctic charr (*Salvelinus alpinus*), European eel (*Anguilla anguilla*); channel catfish (*Ictalurus punctatus)* and Turbot (*Scophthalmus maximus*). These were reported in Baardvik and Jobling, (1990); Kamstra, (1993,) and Sunde et al, (1998) respectively. Therefore grading out fast growers may not be a sustainable and efficient technique for managing growth variants.

Matins (2005) reported that hierarchy is not playing a major role in explaining weight (growth) variation in *C. gariepinus*. The author therefore concluded that individual growth variation in African catfish could have a genetic basis. This calls for a search for genetic explanation on the submission. According to Plomins and Daniels (2011), genetic research seldom finds evidence that more than half of the variance for complex behavioral traits is due to genetic differences among individuals. Mayr, (1969) reported that genetic basis of phenotypic divergence could be established through biochemical analysis of proteins. Biochemical analysis of total protein and isozyme markers has revealed better diagnostic genetic potentials and is usually free from genotype X environment interactions (Lombard et al, 2001; Torkpo et al, 2006). Analysis of genetic and biochemical basis of growth superiority in *C. gariepinus* could therefore be achieved through electrophoresis profiling of their protein using biochemical techniques.

This would be relevant in generating markers for identifying the growth variants and provide information on basis of superiority of the fast growers. It will also contribute to development of sustainable technique for management of growth variants which would lead to increased productivity. Knowing how to manage individual variation in growth and feed intake contributes to maximization of production efficiency (McCarthy et al, 1992; Baardvik and Jobling, 1994); This study therefore assessed biochemical and genetic basis of growth variation in African catfish (*Clarias gariepinus*) for better utilization and management of the variants.

MATERIALS AND METHODS

Experimental Sites

Breeding and generation of growth variant groups was carried out at Fish Laboratory of the Department of Animal Production, College of Agricultural Sciences, Yewa Campus of Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Electrophoresis profiling was carried out at the Central Biotechnology Laboratory of the Federal University of Agriculture, (FUNAAB), Abeokuta, Ogun State, Nigeria.

Experimental Fish

Sample of 90 individuals of a three weeks old *Clarias gariepinus* produced at the Fish Laboratory, College of Agricultural Sciences, Olabisi Onabanjo University, Yewa campus, Ayetoro, Ogun state, Nigeria was utilized for this experiment. The individuals were the products of a breeding exercise in which pooled eggs from three females was mated with a single male donor.

Experimental Design

The individuals were graded to three growth variants groups: fast-S (shooters), average-A, and slow-R growers each containing 30 individuals each (10 individuals in triplicates per treatment) and raised inside a 56.5 x 40.0 x 24.5cm plastic tank filled to 2/3 of its capacity. The variants were described as presented by Nwadukwe and Nana (2000). The fish samples were fed *ad-libitum* twice daily with Copens feed of different size ranges for 3 weeks under the same laboratory condition. At the expiration of three (3) weeks, weight (g) and length of individual fish in each group was taken using digital sensitive scale (Falcon, Bl 3002) and meter rule respectively. Pooled weight and lengths were compared for the groups using one-way analysis of variance (ANOVA). The weights were arranged in hierarchy to select the best and the least four (4) individuals in the S and R groups being the respectively best and least individuals in all the three (3) groups. The eight (8) individuals were subjected to protein extraction and electrophoresis of tissue samples. Steps for analysis of biochemical and genetic basis of growth variation followed standard procedures (Omitogun et al., 1999; Torkpo et al., 2006 and Oyebola et al., 2013).

Tissue Sample Collection

Tissues samples were obtained from the eight (8) selected individuals, conditioned in iced container and transported to the Central Biotechnology Laboratory of the Federal University of Agriculture, (FUNAAB), Abeokuta, Nigeria where protein extraction and subsequent electrophoresis of the samples was carried out. The best four growers were denoted as S group and labeled S_1 to S_4 while the best slow growers were denoted as R group and labeled R_1 to R_4.

Protein extraction

Extraction of soluble protein for Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) followed standard laboratory protocol. Paste of equal quantity of tissue (0.3g) from each of the individuals was obtained and homogenized with extraction buffer (800µl of 0.1M tris-HCl at pH7.6). The paste mixture was vortexed for 1min and rotated at 10,000rpm for 5mins. The supernatant was then collected into a new eppendorff tube and kept at 4^oC. Electrophoresis preparation, electrophoresis conditions, staining and destaining procedures followed Laemmli, (1970). Electrophoresis profiling of the soluble proteins was conducted using gel electrophoresis apparatus (Consort EV 231). The protein extract for all samples was applied to 12.5% poly-acrylamide gel. Dye stocks were stored at 4^0 C and later boiled for 3mins before gel was loaded. 6µl protein sample was added to 3µl of 3x Leammli dye stock. Gel was polymerized in gel caster; a thin layer of isopropanol was added to gel and poured into caster after which wells were created. Prepared samples were loaded in individual wells and run for 2hours at 150V and 0.5 mini-amps inside the electrophoresis machine. Gels were removed from the electrophoresis cell. The resolved images were scanned and stored in computer system. The polyacrylamide resolving and stacking gels for the SDS-PAGE is presented in Table 1 while composition of loading and running gels are presented in Table 2.

Protein Profile Scoring

Data were collected from the produced gel phenogram and used for profile analysis. Bands were viewed and scored based on presence (1) or absence (0) of protein bands. The positions of the proteins as enumerated by Gatehouse (1979) and Machuka (2001) were determined using standard molecular weight proteo-ladder supplied by Norgen Biotec Corp. (www.norgenbiotek.com) and measured in kilo-Dalton (kDa). Bands were presented in increasing order of alphabet following decreasing order of molecular weight of the bands. Divergence of groups at band locus of known biochemical implication was used to establish biochemical differences between groups. Similarities and divergence of individuals band scores were carried out via cluster analysis utilizing UPGMA (Unweighted Paired Group Method of Algorithms) for phenogram grouping (Sneath and Sokal, 1973). This was used to establish phylo-genetic differences between the studied groups. Analysis was done using NTSYS computer software.

RESULTS

Table 3 showed the mean weight and length of the studied growth variant groups in *C. gariepinus*. The result revealed that the S and R groups had significant difference in mean weight and length at $P<0.05$ while the average size group (A) was intermediate. This indicates that the S and R variants are the major factors with respect to growth variation in *Clarias gariepinus.*

The produced dendrogram from SDS-PAGE electrophoresis profiling of the populations is presented in Plate 1. The profile revealed wide range of molecular weight allele and polymorphism of allele across genotypes in the population. The allele had molecular weight range of <14.7 to >150kDa and this was obtained from a total of 19 bands (alphabets A-S). Most of the bands were within 14.7 and 150kDa except band A and R with higher and lower molecular weight (<14.7 and >150kDa respectively). Band C was unique to individual 1 of the S group. Band R which corresponded with

Table 1: Solution for the 6%stacking gels, 12.5%resolving gel for SDS-PAGE

Substance	Resolving gel (ml)	Stacking gel (ml)
Acrylamide bis-acrylamide	3.1	1.0
Tris buffer (1.0M Tris-HCl, pH8.8)	3.0	0.63
20% (w/v) SDS	0.038	0.025
dH_2O	1.30	3.6
10%APS (Ammonium persulphate)	0.036	0.025
TEMED (Tetramethylenediamine)	0.01	0.01

Table 2: Composition of Solution for Loading and Running Buffers of Gel

Loading buffer (Laemmli Loading dye)(3x stock)		Running buffer(Laemmli buffer) (10x)	
1M Tris-HCl pH6.8	(4ml)	Tris base	(30.3g)
20%SDS	(3ml)	Glycine	(144.0g)
100% Glycerol	(3ml)	SDS	(10.0g)
Bromophenol blue	(0.006g)		
Make up to	10ml	dH_2O make to	1liter

Table 3: Mean Length and Weight gain of shooters (S), average (A) and runts (R) Population at 6 weeks rearing period

	S	A	R
Length	5.82 ± 2.73^{a}	2.98 ± 1.96^{ab}	1.26 ± 1.53^{b}
Weight	4.16 ± 2.40^{a}	2.52 ± 2.47^{ab}	0.59 ± 0.83^{b}

*Means with same subscript along rows are not significantly different

<14.7kDa molecular weight and band A of >150kDa molecular weight were solely inherited by S group members; 100% of the members inherited band R while 75% (3out of 4 individuals) of the members inherited band A. However, one (1) out of the four (4) individuals in S group did not inherit allele A. Meanwhile 100% of the individuals in R growth group (all individuals) did not inherit both allele A and S.

Analysis of the band scores presented in Table 4 revealed that 88.82% of the 152 allelic sites inherited protein bands, 21.05% out of the 19 bands were polymorphic while frequency of occurrence of each of the bands was between the range of 0.13 and 1.00. Dendrogram of the UPGMA similarity matrix of the individuals is presented in Figure 1. Analysis of the dendrogram showed that at 0.75 coefficient of similarity, two major clusters were generated; one cluster distinctively had 75% of S group members (3 out of 4individuals). The rest 25% (one individual) was closest to the rest of its group members but located on a sub-branch of the second cluster thus indicating discrimination between S and R sub-groups as well as variability within S- group members

DISCUSSION

Growth performance and fry survival of African catfish fry is affected by feed (Musa et al. 2012). However, under the same feeding condition, grades of sizes of the bred fish were obtained in the current study. This indicates that feed cannot be the outright factor responsible for growth variation in bred C*. gariepinus* populations. The obtained growth variants of the S and R represent hierarchical growth (size) groups which could generate challenges of social hierarchies. According to Metcalfe et al. (1995) and Metcalfe (1998), social hierarchy has been identified as the most important factor that is responsible for growth variation in group-housed fish. In practice, management of S and R groups constitute challenges in *Clarias gariepinus* hatchery. The R group members are usually managed by combining it with the average sized group (group A members). This is because, in most cases, members of A group forms the majority of offspring

Table 4: Distribution of Identified 19 bands across the electrophoresed samples of eight (8) individuals in the growth variant populations.

Allele bands	Number of occurrence 19 bands	Frequency
A	3	0.38
B	8	1.00
C	1	0.13
D	8	1.00
E	8	1.00
F	8	1.00
G	8	1.00
H	8	1.00
I	7	0.88
J	8	1.00
K	8	1.00
L	8	1.00
M	8	1.00
N	8	0.50
O	8	1.00
P	8	1.00
Q	8	1.00
R	8	1.00
S	4	0.50

population in a batch; the R group members are of sizes that are too big for the average sized group (group A members) to prey on and will stand better with respect to competition with the group A member than when kept with the S group members. The insignificant difference between the R and A as obtained in the current study, corroborate the management technique of pooling group A with R for onward rearing while fast growers (group S) are sorted out. This thus supports Cutts et al. (1998) who reported that grading and discarding of fast growers (shooter referred to as S group members in this study) are preferred in commercial fish production. Hence, the result of the current study may be underscoring the relevance of sorting out the S group from a bred fish batch for better production gains.

The activities of the S group members seem to place some challenges on them. According to Uka et al. (2005), fast growers have cannibalistic tendency on the slower growing ones and this has an attendant negative effect on yield and final remuneration from the industry. Explanation on tendencies for the negative challenges of the fast growers seems to stem out of feeding behavior and territorial aggression. Fish often defend their food and territory (Ruane, 2000). These activities have been argued to have origin in the need to balance the need to eat and not to be eaten (Thorpe and Cho, 1995) and it has led to aggression and cannibalism which results in skin lesions, fin damage and in extreme cases, mortality (Solomom and Udoji, 2011). In most cases, it is opined that presence of social hierarchy in a batch of bred fish is a potential source of mortality and possibly slow growth of the subordinates of the fast growers. This idea is supported by the concept that larger fish would have greater aggressive and suppressive effect on growth and feed intake of the subordinate (smaller) fish. Therefore the fast growers are usually removed and discarded. However, growth rate of the S group's member is worth to be investigated and utilized in aquaculture. According to Mahapatra (2008), growth rate, survival and disease resistance are the most important production traits. In an earlier discussion in the current study, it has been observed that apart from social hierarchy, some other factors could be important in giving explanation on basis of growth variation in the bred fish population. An understanding of the basis of growth superiority of the S group members may reveal potential importance and subsequent utility of the variants other than discarding them. The electrophoresis of protein samples of the S and R groups as carried out in this study revealed this information.

Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS PAGE) showed polymorphism of protein bands across genotypes of *C. gariepinus* growth variant sub-groups. Bands were private to individual at locus C; sub-groups at locus R while several homologous sites were observed with respect to the population. This indicates the suitability of the technique in establishing polymorphism in the studied population. SDS PAGE analysis has been found useful in establishing genetic and biochemical relationship in species of plant and fish origin (Omitogun et al., 1999; Torkpo et al., 2006 and Oyebola et al., 2013). Most of the bands were within

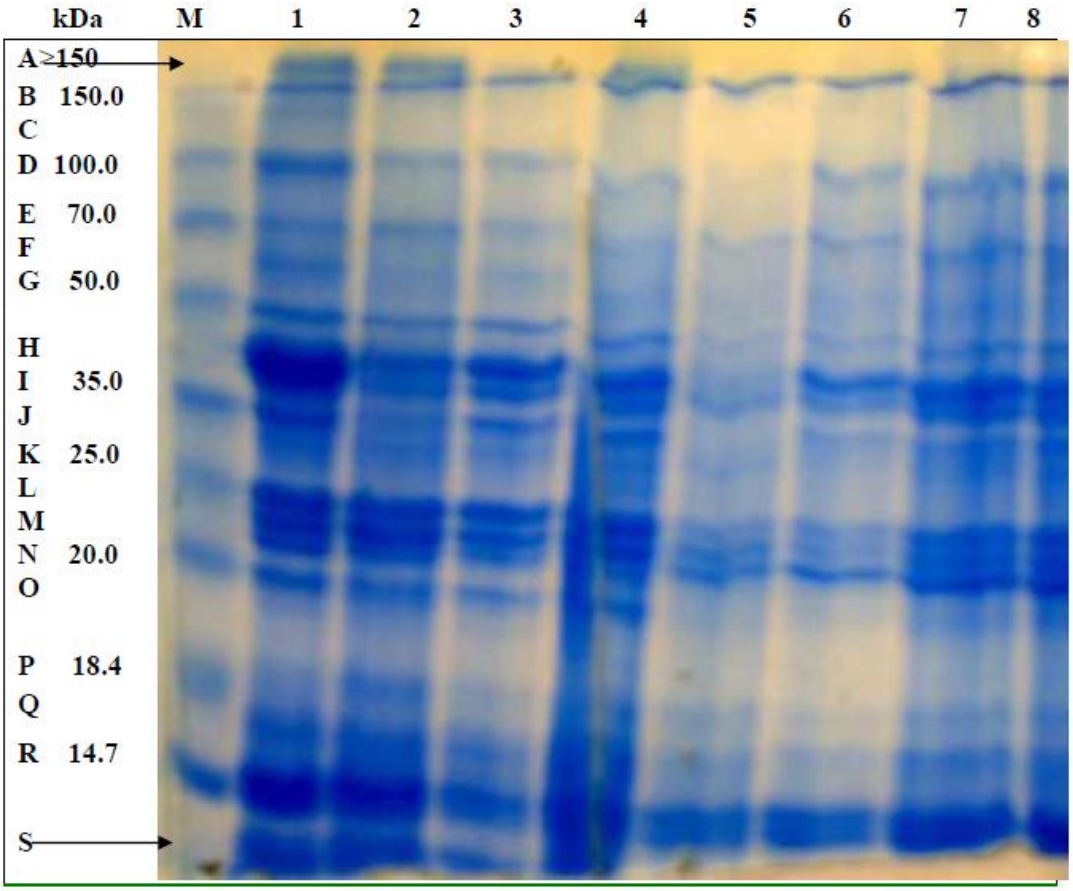

Figure 1: Gel phenogram of the samples of the eight (8) electrophoresised *Clarias gariepinus* individuals.
***Column 1-4 belong to S- growth group while 5-8 belong to R-growth group; M=markers bands, Alphabets A-S= generated band locus**

medium molecular weight range: 14.7 and150Kda, while a <14.7kDa and >150kDa molecular weight bands (A and R) were also visible. These bands were in the range of low and high molecular weight bands. The presence of low, medium and high molecular weight bands in this species could account for high level of variability and thus versatility of the species. *Clarias gariepinus* possesses versatile locomotory behaviour (Gunder, 2004), easily adapted to environment where the temperature is higher than 20^0C (FAO, 2012), has versatile adaptive features and can adapt to interspecific competition and predation pressures through body size, shape, head protection, pectoral spines and piscivorous habits (Bruton, 1979).

Protein bands A with the highest molecular weight and band R with the lowest molecular weight could be seen as alleles that are of genetic and biochemical importance encoding for protein differences and growth performance between and within the sub-groups. The bands were solely inherited by 100% and 75% of S group members respectively and were not inherited by any member of the R growth group. The sole inheritance of the highest and lowest molecular weight band by the S group indicates that the groups are more versatile. Hence, they would take advantage of this in culture environment. The allele R could be insinuated as the potential genetic site for discriminating the growth variant group members as it distinctively separated the two sub-groups. Figure 2

Gel electrophoresis has the advantage that it can directly equate variation in protein banding patterns to genes encoding these proteins (Gottileb, 1971).Band R and A corresponded with <14.7kDa and >150kDa molecular weight respectively. Band R could be biochemically and genetically linked with α-amylase inhibitor and and a polymorphic form of prostaltic growth factor respectively. Gatehouse et al., (1979) and Machuka (2001) determined position of proteins using low and high molecular weight markers in Kilodalton such as: phosphorylase B, 94; bovine serum albumin,

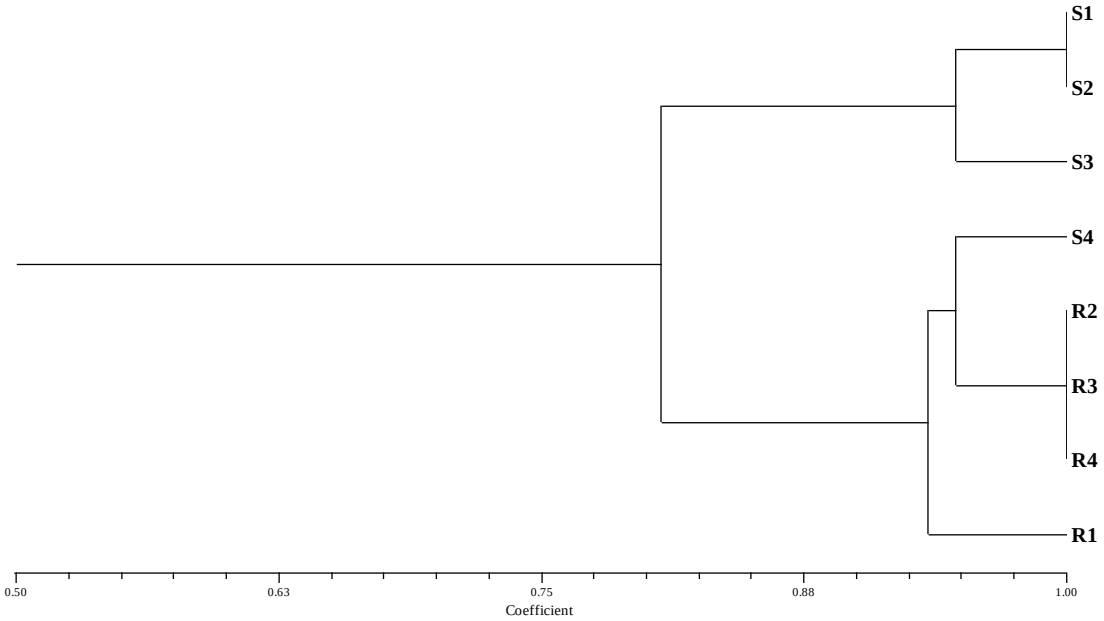

Figure 2: Dendogram for Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis of eight (8) *Clarias gariepinus* individuals belonging to the S (shooters) and R (runt) growth variant populations.

67;ovaalbumin, 43;carbonic anhydrase, 30;trypsin inhibitor, 20.1 and α–amylase inhibitor, 14.4 while Chevalter et al., (1993) linked 150kDa molecular weight with a major prostatic growth factor (PGF-1). The position of allele R was observed to be closest to 14.4 which correspond to the molecular weight of α –amylase inhibitor. This protein inhibitor is believed to make plants less palatable and even lethal to insects (Sasikiran et al., 2002) and it has possibility of being useful in treating obesity and diabetes mellitus resulting from defects in insulin secretion (Ali et al., 2006). Proteinase inhibitors are a potential model system that is used to study basic evolutionary processes, such as functional diversification (Christeller, 2005). Meanwhile Oyebola et al., (2013) reported existence of functional disparity in pectoral spine sub-species of *C. gariepinus*; the sub-species genotypes were 100% canonically discriminated and were biochemically separated by the presence or absence of alpha-amylase inhibitor. According to the authors, specimens that possessed smooth anterior portion of pectoral spines were those that inherited the band that corresponded to the biochemical compound. Hence, growth superiority as observed in the current study could be linked with the mixture of genetic materials that originated from parents of the different sub-species for the breeding exercise that generated the population.

Position of allele A was closest to 150kDa and could therefore be insinuated as a polymorphic form of 150kDa band. This band was linked with a major prostatic growth factor (PGF-1) for androgen independent prostatic epithelial cells in dog serum (Chevalter et al., 1993).This indicates that it could have contributory role in the groups growth performance. Allele A seems to be another site for discrimination among the fast growers. This is because, within the subgroups variability was observed in it. Although, both allele A and R were inherited by the S subgroup while the R subgroup members did not; band A was inherited by 75% of S group members unlike in allele R which was inherited by all the group members. This indicates within sub-group variability of the S group. Cluster analysis of the scored alleles also supported the pattern of similarity of the members of the groups as all members of each of the groups were closest to each other but distant to the other group. The cluster dendrogram using Jaccard's coefficient of similarity of scored alleles (Jaccard, 1901) generated two major clusters; one of the clusters accommodated three (3) out of the four analyzed members of S group; the fourth individual being the closest to it on the second cluster. Hence, growth variation of significant importance in *C. gariepinus* as it was observed in this study has biochemical and genetic basis. The S and R groups were discriminated at both biochemical and genetic levels and specific sites for their identity was highlighted in this study.

Genetic research seldom finds evidence that more than half of the variance for complex behavioral traits is due to genetic differences among individuals (Plomins and

Daniels, 2011). A genetically different individual would have differential phenotypic attributes in different environment. One of the sites for discriminating the S and R groups was linked with sub-species while the other showed within S group variability that is of growth implication. A mixture of genetic materials from individuals possessing allele R with those that did not possess it would result in heterosis, hence the production of heterogeneous progeny population. The allele R was also linked with high versatility which would result in differential performance in culture environments as well as adaptability. It was also observed to have relationship with allele A which could be linked with a growth factor; hence growth variation would also result in such progeny population. Breeding a pooled population of *C. gariepinus* should take cognizance of subspecies status of broodstocks in order to reduce the menace of abnormal growth variations.

The obtained knowledge could be used in discriminating between fast and slow growers, aside this; genetic discrimination of cultivars is of importance in breeding and stock improvement. Omitogun et al (2001) observed that genes controlling each character can be mapped and isolated to complement and hasten the work of breeders for genetic improvement. Hence, the relevance of the results of this study in genetic improvement via marker assisted selection (MAS), and planning of breeding programmes in *C. gariepinus* species.

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***ABBREVIATION**

S- Shooters or fast growers
R-Runt or slow growers
Size group (A) - Intermediate weight size between S and R
SDS-PAGE- Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
kDa - kilo-Dalton
PGF-1- Prostatic Growth Factor-1
MAS- Marker Assisted Selection
UPGMA- Unweighted Paired Group Method of Algorithms

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