MOLECULAR SEQUENCE-BASED POLYMORPHISMS IN THE INSULIN LIKE GROWTH FACTOR (IGF-I) OF NILE TILAPIA (*Oreochromis niloticus*) FROM DIFFERENT WATER BODIES

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Abstract
In recent years, salinity levels in the Senegal River Delta have been altered by sea-level changes with significant transgressions and regressions which has had a severe impact on freshwater fish farm productions throughout the region. The high salt in a tilapia fish farm location is considered potentially stressful to fish and, therefore, predisposing to pathologies and slow growth. This study was aimed at identifying the variability and assessing the novel nucleotide and amino acid polymorphism present in the growth gene of Oreochromis niloticus from the two West African sub-regions (Nigeria and Senegal). All individual samples from Epe river, Nigeria (ONN), and Senegal river basin by Diama (ONS) were screened for genetic variation by a gene-specific PCR reaction with the primers (IGF-I forward-5'-CTTGGACGAGTAGGAGGCAAATG-3'and IGF-I reverse- 3'-AAATACAAGCAAGCGATAAGAA-5') of 447 bp (IGF-I, GenBank accession AF033797). Nucleotide and amino acid (AA) sequences obtained were edited and aligned and a phylogram was created using Clustal O (version1.2.4) multiple sequence alignment software. High polymorphisms were detected for both the nucleotides and amino acids on selected strains of the tilapia and the phylogenetic tree generated revealed lower similarities between ONN and ONS and higher similarities among ONN1 and ONN2 and ONS1 and ONS2. This study provides additional information regarding variation in insulin growth factor I of O. niloticus that could promote association study for production traits.
Keywords: Salinity**,** Insulin-like growth factor I (IGF-I), nucleotide polymorphism, amino acid polymorphism, *Oreochromis niloticus* Nigeria (ONN), and *Oreochromis niloticus* Senegal (ONS).

INTRODUCTION

Tilapia is not only widely accepted to African consumers but also other parts of the world as it represents the species of choice due to its high growth rate, great resilience to environmental stresses, ease of reproduction, and unquestionable market demand. Nile tilapia (*Oreochromis niloticus*) is considered the leading tilapia species. Although this species is native to Africa (Trewavas, 1983; McAndrew, 2000) it has great economic significance to the global fisheries and aquaculture sector (Nyingi *et al*., 2009 Fatsi *et al*., 2020). Therefore, its contribution demands research activities in different areas of aquaculture (Shimaa *et al.,* 2014 and Esmail, 1997). The successful commercial culture of Nile tilapia has previously been attributed to its robust adaptiveness to new environments (Aloo, 2003) and different environmental conditions (Firmat *et al*., 2013). Its populations have been observed to rapidly adapted to various environments outside its native home of Africa by evolving outstanding ecological and physiological features, making them highly successful invaders throughout the tropical and subtropical regions (Trewavas, 1983; Canonico *et al*., 2005; Firmat *et al*., 2013), and have even been shown to adapt to extreme environments (Nyingi *et al*., 2009; Ndiwa *et al*., 2014).

Oreochromis niloticus as a Cichlid are well known for their complex genetic structure (Kaufman, 1992; Meyer, 1993; Abila *et al*., 2004; Kerschbaumer and Sturmbauer, 2011; Hashem *et al*., 2020). Some important variables considered crucial for species and population diversity include variations in phenotypic features, gene expression regulation, and differential transmission of allelic combinations. (Gagnaire *et al*., 2013, 2015); principally, however, ecological diversity is a prime factor bonding the environment, selection pressure, and species peculiarity during diversity (Gagnaire *et al*., 2013). Over the past millennia, sea-level changes with widespread transgressions and regressions have affected salinity levels across major Senegal Rivers (Diaw *et al*., 2020) and this has had an adverse effect on seafood production in the region (Dioufand Albaret, 1996).

As a result of the sudden higher salinity difference observed and the subsequent decline in fish farming activities of certain parts of Senegal and the unusual survival of *Oreochromis niloticus* strains in these regions, we decided to employ the use of IGF-1 factor to examine the genome of this species by profiling its nucleotide and amino acid. Although the use of genomics in aquaculture is still in its early stages, the use of Single Nucleotide Polymorphisms (SNPs), which were used in this study, has become a focal point in molecular marker development because they are the most abundant polymorphism in any organism, are adaptable to automation, and reveal hidden polymorphism not detected by other markers and methods (Hecker *et al*., 1999). SNPs are most reliable for genomic-wide association studies (GWAS) because linkage instability can be detected with high-density SNP

coverage of the genome when working with production or performance traits as it is in the present study.

The IGF-1 factor is known to encode growth proteins (Cuevas-Rodríguez *et al..*, 2016), which are vital to the significant growth rate difference between Nile tilapia and other tilapia fish species Sokenu *et al.*, 2020).

MATERIALS AND METHODS

Fish Sample collection and identification

Live tilapia fishes comprising 20 units of *Oreochromis niloticus* from Nigeria (ONN) and 20 *Oreochromis niloticus* from Senegal (ONS) were sourced from Epe river, Epe (latitude 6º35'2" N and longitude 3º59'0" E), Lagos State, Nigeria, and Senegal river basin by Diama dam (15°400 W and latitudes 15°450 N), Senegal respectively. The caudal fins of the fish samples were clipped and placed in separate plain 10ml sterile tubes each containing 4ml absolute ethanol.

Identification of the fish samples was achieved from the description checklist and identification keys (FAO, 1996; Froese and Pauly, 2003).

DNA extraction and amplification

On the bench at the biotechnology laboratory of NIOMR, Badore outstation, Nigeria, genomic DNA was extracted from the caudal fin tissue of the fish samples using a modified chlorophenol/isoamyl/alcohol procedure, as described by Sambrook and Russell (2001). The integrity of the DNA was checked on 1% ethidium bromide-stained agarose gel electrophoresis and the isolate was stored at -20ºC prior to PCR amplification.

All individuals were screened for genetic variation by a gene-specific PCR reaction with the primers (IGF-I forward- 5'-CTTGGACGAGTAGGAGGCAAATG-3' and IGF-I reverse- 3'-GAAATACAAGCAAGCGATAAGAA-5') of 447bp (IGF-I, GenBank accession AF033797). The DNA amplification was carried out following Bui *et al.*, 2017.

Nucleotide and amino acid sequencing

4 of the amplicons from both water bodies were selected from the amplification of the *O. niloticus* (2 from Nigerian water (ONN 1 & ONN2) and 2 from Senegal water (ONS1 & ONS2) which were bidirectionally sequenced in an automatic sequencer (ABI 3500XL Genetic Analyzer).

Nucleotide sequences obtained were edited and aligned and a phylogram was created using Clustal O (version1.2.4) multiple sequence alignment software. The nucleotide Polymorphisms were discovered by visual analysis while the amino acid sequence was obtained by translation of the DNA sequence of each species using biolign alignment software (version 4.0.6.2) and the amino acid sequence was aligned, and their Polymorphisms were analyzed using Clustal O (version1.2.4) multiple sequence alignment software. A polymorphic position was defined as a real polymorphism when it was present in more than one individual and was confirmed by the forward and reverse sequence.

RESULTS

DNA extraction and amplification

The amplified PCR product of the extracted DNA run on 1.5% ethidium bromide-stained agarose gel demonstrated that IGF-I genes had the same bands which demonstrated equal length and size of the gene in the Tilapia fishes' samples as shown on *plate 1, where M is the known 50bp- 10 kb DNA ladder.*

The results of the analysis are presented in the tables and figures below.

CLUSTAL O (1.2.4) multiple sequence alignment Nucleotides (Forward)

```
ONN1F
GTTTTTTCTTTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC    60
ONN2F     ----------ATGGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC 47
ONS1F     ----------TGTCCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC   46
ONS2F                                                               --------
CTTTTAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC      52
                ****************************************
           ************************************************************
ONN1F     ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGCTTTGTATTTCA   412
ONN2F     ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCA   399
ONS1F     ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGCTTGTATTTCA -
          397
ONS2F     ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGCTTGTATTTCA -  403
          ************************************** **   **
```

Figure 1: Part view of sequence alignment of the forward reaction of the IGF-1 of *Oreochromis niloticus* from Nigeria and Senegal.

* Means similarities among the sequence bases of the four fishes.

CLUSTAL O (1.2.4) multiple sequence alignment for Nucleotides (Reverse)

```
ONS1R     -TTTTTTTTCTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGAAT
59
ONS2R
TTTTTTTTTTTTTTTTAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGAAT      60
ONN1R                                                              -
TTTTTCTTCTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGAAT       59
ONN2R
TATTGGTTTCTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGAAT      60
          **   ** ***** ******************************************
           ************************************************************
ONS1R
GACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTACTTCGTCCAAGA            414
ONS2R
GACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTACTTCGTCCAAGA            415
ONN1R          GACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-
           413
ONN2R          GACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTATTCGTCCAAGA-
           414
          ****************************************** *   * *
```

Figure 2: Part view of the alignment of the reverse reaction of the IGF-1 of *Oreochromis niloticus* from Nigeria and Senegal showing polymorphism.

* means similarities among the sequence bases of the four fishes.

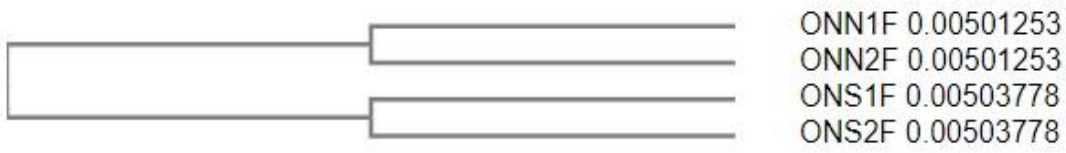

Figure 3: Phylogenetic tree of the DNA sequence from IGF-I of *Oreochromis niloticus* from Nigeria and Senegal.

```
>ONN1F

1      GTT TTT TCT TTT GAA TGT CTG TGT AAT GTA GAT AAA TGT GAG GGA    45
1       V   F   S   F   E   C   L   C   N   V   D   K   C   E   G    15

46     TTT TCT CTC TAA ATC CGT CTC CTG TTC GCT AAA TCT CAC TTC TCC    90
16      F   S   L   *   I   R   L   L   F   A   K   S   H   F   S    30

91     AAA ACG AGC CTG CGC AAT GGA ACA AAG TCG GAA TAT TGA GAT GTG    135
31      K   T   S   L   R   N   G   T   K   S   E   Y   *   D   V    45

136    ACA TTG CCC GCA TCT CAT CCT CTT TCT CCC TGT TTT TAA TGA CTT    180
46      T   L   P   A   S   H   P   L   S   P   C   F   *   *   L    60

181    TAA ACA AGT TCA TTT TCG TCG GGC TTT GTC TTG TGG AGA CCC GTG    225
61      *   T   S   S   F   S   S   G   F   V   L   W   R   P   V    75

226    GGG ATG TCT AGC GCT TTT TCC TTT CAG TGG CAT TTA TGT GAT GTC    270
76      G   M   S   S   A   F   S   F   Q   W   H   L   C   D   V    90

271    TTC AAG GTA ACT TAC CTG ATT TCC TTT GAC ACT ATA CAT TAT CAC    315
91      F   K   V   T   Y   L   I   S   F   D   T   I   H   Y   H   105

316    CTT GAT TCT TCA CTT GCT CAC TAT TTG CAC AGA GCA TCC TCG CCT    360
106     L   D   S   S   L   A   H   Y   L   H   R   A   S   S   P   120

361    ACT TTA AAA AGA AAC AAT AAA AGG GGA TTC TTA TCG CTT GCT TTG    405
121     T   L   K   R   N   N   K   R   G   F   L   S   L   A   L   135

406    TAT TTC   411
136     Y   F
```

Figure 4: Translation of DNA sequence of IGF-1 of *Oreochromis niloticus* from Nigeria (ONN) to amino acid.

* means stop codon.

```
>ONS2F

1      CTT TTA ATG TCT GTG TAA TGT AGA TAA ATG TGA GGG ATT TTC TCT    45
1       L   L   M   S   V   *   C   R   *   M   *   G   I   F   S    15

46     CTA AAT CCG TCT CCT GTT CGC TAA ATC TCA CTT CTC CAA AAC GAG    90
16      L   N   P   S   P   V   R   *   I   S   L   L   Q   N   E    30

91     CCT GCG CAA TGG AAC AAA GTC GGA ATA TTG AGA TGT GAC ATT GCC   135
31      P   A   Q   W   N   K   V   G   I   L   R   C   D   I   A    45

136    CGC ATC TCA TCC TCT TTC TCC CTG TTT TTA ATG ACT TTA AAC AAG   180
46      R   I   S   S   S   F   S   L   F   L   M   T   L   N   K    60

181    TTC ATT TTC GTC GGG CTT TGT CTT GTG GAG ACC CGT GGG GAT GTC   225
61      F   I   F   V   G   L   C   L   V   E   T   R   G   D   V    75

226    TAG CGC TTT TTC CTT TCA GTG GCA TTT ATG TGA TGT CTT CAA GGT   270
76      *   R   F   F   L   S   V   A   F   M   *   C   L   Q   G    90

271    AAC TTA CCT GAT TTC CTT TGA CAC TAT ACA TTA TCA CCT TGA TTC   315
91      N   L   P   D   F   L   *   H   Y   T   L   S   P   *   F   105

316    TTC ACT TGC TCA CTA TTT GCA CAG AGC ATC CTC GCC TAC TTT AAA   360
106     F   T   C   S   L   F   A   Q   S   I   L   A   Y   F   K   120

361    AAG AAA CAA TAA AAG GGG ATT CTT ATC GCT TGC TTG TAT TTC   402
121     K   K   Q   *   K   G   I   L   I   A   C   L   Y   F
```

Figure 5: Translation of DNA sequence of IGF-1 of *Oreochromis niloticus* from Senegal (ONS) to amino acid. * means stop codon.

```
ONN1F                                                                VFSFECLCNVDKCEGF--
SL*IRLLFAKSHFSKTSLRNGTKSEY*DVTLPASHPLSPCF*     55
ONN2F                                  ---MVCVM*INVRDFLSKSVSCSLNL-------------------------TSPKRAC--
                                    29
ONS1F     -----------------CPV*CR*M*GIFSLN-------------------PSPVR*I--                          18
ONS2F     ---------------LLMSV*CR*M*GIFSLN-------------------PSPVR*I--                          20
                    :                 *
ONN1F     *L*TSSFSSGFVLWRPVGMSSAF--------S----------------------FQWHLCD                        84
ONN2F                                                                ----AMEQS-----RNIEM*HCP------
HLILFLPVFNDFKQVHFRRALSCGDPWGCLA                    73
ONS1F     ----SLLQNEPAQWNKVGILRCDIARISSSFSLFLMTLNKFI------------------                          56
ONS2F     ----SLLQNEPAQWNKVGILRCDIARISSSFSLFLMTLNKFI------------------                          58
          : ..    .:: .
ONN1F                                        VFK----------------------VTYLISFDTIHYHLDSSLAHYL-----------
HR                                 111
ONN2F                           LFPFSGIYVMSSR*L-T*FPLTLYIITLILHLLTICTEHPRLL*KETIKGD---------
                             120
ONS1F                                                                 ---FVGLCLVETRGDV*RFFLSVAF------
M*CLQGNLPDFL*HYTLSP*FFTCSLFAQ                      103
ONS2F     ---FVGLCLVETRGDV*RFFLSVAF------M*CLQGNLPDFL*HYTLSP*FFTCSLFAQ
          105
                              : : .   *:
ONN1F     ASSPTLKRNNKRGFLSLALYF                                                                  132
ONN2F     ------------SYRLFCIS-                                                                  128
ONS1F     SILAYFKKKQ*KGILIACLYF                                                                  123
ONS2F     SILAYFKKKQ*KGILIACLYF                                                                  125
            .   .:
```

Figure 6: Alignment of the amino acid sequence of the IGF-1 of *Oreochromis niloticus* from Nigeria and Senegal. * means stop codon.

Table 1: Observed *polymorphisms between the forward nucleotide sequence reaction of the Oreochromis niloticus*.

POSITION	ONN	ONS
404	T	G
405	G	T
406	T	A
407	A	T
410	T	C
411	C	A

Table 2: ***Observed polymorphisms between the reverse nucleotide sequence reaction of the Oreochromis niloticus.***

POSITION	ONN	ONS
404	T	C
406	C	T
407	G	C
408	T	G
409	C	T
414	A	G

Table 3: Detected P*olymorphisms in the amino acid sequence of the Oreochromis niloticus.*

SEQUENCE POSITION	ONN	ONS
24	L-	*
58	C--	I-
69	S--	N--
75	R-	N--
79	M-	I-
154	T--	C--
155	I-	L-

* means stop codon; - means Essential AA; -- means Nonessential AA

Nucleotide and amino acid sequence and analyses

The sequence alignment generated for the forward and reverse primers of IGF-1 of the four *Oreochromis niloticus* from Nigeria and Senegal water bodies were shown in Figure 1 and 2 respectively while Tables 1 and 2 shows the nucleotide polymorphisms detected at positions 404(T/G), 405(G/T), 406(T/A), 407(A/T), 410(T/C) and 411(C/A) of the forward reaction sequence and those detected in the reverse reaction sequence at positions 404(T/C), 406(C/T), 407(G/C), 408(T/G), 409(C/T), 411(A/C),413(G/A), and 414(A/G) respectively.

The phylogenetic tree revealed lower similarities between ONN and ONS and higher similarities among ONN1 and ONN2 and ONS1 and ONS2 (Figure 3).

A total of 137 and 134 amino acid sequences were translated from the DNA sequence of *Oreochromis niloticus* from Nigeria and Senegal respectively as shown in Figures 4 and 5. The alignment of these sequences revealed seven (7) Variations at positions 24, 58, 69, 75, 79, 154, and 155 as shown in Figure 6.

The polymorphisms observed are as follows; position 24; C (cysteine) in ONN to I (isoleucine) in ONS, position 58; L (leucine) in ONN to *(stop codon) in ONS, position 69; S (serine) in ONN to N (asparagine) in ONS, position 75; R(arginine) in ONN to N(asparagine) in ONS, position 79; M(methionine) in ONN to I (isoleucine) in ONS; position 154; T(threonine) in ONN to C(cysteine) in ONS and position 155; I (isoleucine) in ONN to L(leucine) ONS (Table 3)

DISCUSSION.

The DNA sequences obtained from the primers employed in this investigation were around 447bp, which is consistent with the findings of Cuevas-Rodrguez et al., (2016) and Sokenu et al., (2020). This study was aimed at identifying and developing novel nucleotide and amino acid polymorphism present in the growth gene of *Oreochromis niloticus* from the two West African regions (Nigeria and Senegal). These polymorphisms might be responsible for differential growth and large sizes of *Oreochromis niloticus* in these two regions coupled with the different salinity of their water bodies. The lower similarity between ONN and ONS might imply a high genetic polymorphism and a clear indication of their different sources and ancestry. Such genetic markers can be screened in natural population studies of adaptation and associate them to phenotypic polymorphisms in fish evolution.

In this study, sequence alignment of *Oreochromis niloticus* IGF-1 gene was used to investigate and validate genetic variation and single nucleotide polymorphism associated with differential growth traits in tilapia sourced from different environmental conditions. Previous studies have confirmed this candidate gene approach as a strong technique for the identification of polymorphisms in genes that associate with economic traits (Hemmer-Hansen *et al*. 2011, Ukenye *et al.*, 2020). The present study revealed the existence of 12 SNP in contrast to 34 SNPs detected in the coding and noncoding region across the four populations of *Tilapia guinnensis* which is an indication of genetic polymorphism in fish populations. The current finding aligned with the

earlier reports of Cuevas-Rodríguez *et al*., (2016) on the novel single nucleotide polymorphisms in candidate genes for growth in tilapia (*Oreochromis niloticus*) and the 22 SNPs associated with growth in rainbow trout as reported by Salem *et al*. (2012).

The observed SNPs show the diversity in the populations of *O. niloticus* from the Nigerian water body and the Senegal saline basins. Senegal population displayed the most diversity among the two populations as demonstrated by the number of SNP loci and polymorphic sites. This suggests that there is diversity in IGF-1 gene in the Nigeria and Senegal populations of *O. niloticus.* A similar observation was made by Ukenye *et al*. (2016) on genetic diversity assessment of *T. guineensis* using microsatellite markers where Badagry population exhibited high genetic diversity. The relevance of these detected polymorphisms in nucleotide sequence of *O. niloticus* from the two environments could thereby justify the Roman (2018) assertion that if an adequate number of SNPs is identified and used, mutations in genes are associated with a trait can be detected and used in marker-assisted selection.

The work of Shunsuek *et al*., 2000, that detected the involvement of IGF-1 in the regulation of protein, lipid, carbohydrate, mineral metabolism in the cells, differentiation, and proliferation of the cells and ultimately body growth further makes the detected polymorphism in amino acid (AA) sequence of the studied *O. niloticus* in this study very relevant. The current study shows 13 AA sequence polymorphism for both Nigeria and Senegal sourced *O.niloticus* strains. A deficiency, or imbalance, in functional AA, may impair body metabolism and homeostasis as opined by Espe *et al*., (2014) making the interest in AA sequence polymorphism the desired information to increase disease resistance, immune response, reproduction, behavior, and more to fish culture. It was observed that Nigeria sourced *O. niloticus* (ONN) showed 7 polymorphic AA with four being essential amino acids (EAA) while Senegal strain displayed 6 polymorphic AA with only 3 being EAA making ONN being more polymorphic with a higher number of polymorphic AA and EAA. Li *et al*., 2021, has provided compelling evidence that an adequate supply of both traditionally classified nutritionally essential amino acids (EAAs) and non-essential amino acids (NEAAs) in diets improve the growth, development, and production performance of aquatic animals (e.g., larval metamorphosis) hence the contribution of these findings. The relevance of AA polymorphism in fish species diversity was further enshrined by the findings of Bower and Johnston (2010) which indicate that amino acids alone are sufficient to stimulate myogenesis in myoblasts and that IGF-I production is controlled by both endocrine and paracrine pathways which are useful markers for the breeding program.

In conclusion, the polymorphisms identified in the current study indicated some level of genetic variability; hence, SNPs identified in the *O. niloticus* IGF-1 gene contributed to the genetic variation in *O. niloticus* populations of Nigeria and Senegal populations. At present, limited knowledge is available about either the cell- and tissue-specific metabolism of AAs and utilization of AAs in different fish species. These issues should be addressed to develop environment-friendly aquafeeds and reduce feed costs to sustain global aquaculture. The nucleic and AA sequence polymorphism should be further investigated through association study to detect their production potential which may inform useful selection markers for breeding programs.

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