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Comparative study of single nucleotide polymorphisms (SNPs) of a candidate growth gene (IGF-I) in *Oreochromis niloticus* and *Sarotherodon melanotheron*

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In teleost fishes, the regulation of growth performance by the GH and IGF system also seems to be highly conserved. Adult *Oreochromis niloticus (Nile* tilapia) could reach up to 60 cm maximum length while *Sarotherodon melanotheron* (black chin Tilapia) has a maximum length of 28 cm. This study describes the analysis of Single Nucleotide Polymorphism in isolated, amplified, and sequenced DNA from two common Tilapia species (*O. niloticus* and *S. melanotheron)* with the aim of identifying genetic variation and single nucleotide polymorphisms (SNPs) in one of the main genes, Insulin like growth Factor-1 (IGF-I), related to growth in the Tilapia species. The extracted DNA from the clipped caudal fins of the Tilapia species samples using Sambrook and Russell's modified chlolorophenol/isoamyl alcohol DNA extraction method were further amplified in a thermal cycler with designed IGF-I forward and reverse primers of 447 bp which were subsequently sequenced with an automated analyzer. The PCR product was separated on 1.5% ethidium stained agarose gel electrophoresis and the bands revealed on the gel were all of the same length (447 bp). The Sequence alignment revealed a total of five single nucleotide polymorphisms which were detected in the forward reaction at the positions 181, 199, 328, 362 and 369 of the sequences and in the reverse reaction at positions 18, 20, 54, 183 and 201. A total of 138 amino acid sequence was translated from the DNA sequence with variations sequence at positions 1, 3, 4, 61, 67, 121 and 123. These results showed variations among these two fish species which could explain differential growth performance between them.

Key words: Single nucleotide polymorphism (SNP), *Oreochromis niloticus* (ON), *Sarotherodon melanotheron* (SM), Tilapia and insulin-like growth factor (IGF).

INTRODUCTION

Single nucleotide polymorphism (SNP) markers are the method of choice for genetic analyses including diversity and quantitative trait loci studies (Thu et al., 2017). Determination of the genetic variability was described by Lupchinski Jr et al. (2011) as an essential step for the implementation of genetic improvement programs that

are focused on the selection of faster growing fish with lower feed conversion rates and resistant to diseases. Studies of genetic diversity at DNA level represents in expansion field in aquaculture aimed at finding out those DNA variations associated with productive phenotypes, so as to use them as tools for assisting the offspring selection at early stage and possibly predict their performance (Na-Nakorn and Moeikum, 2009). This strategy is known as gene-assisted selection (GAS) (De-Santis and Jerry, 2007). Growth performance is often used as an indicator of the status of individuals and populations in culture and the wild, and therefore, major effort has been applied towards garnering a more comprehensive understanding of how multiple components of the GH (growth hormone) and IGF (insulin-like growth factor) system interact to control growth and metabolism (Picha et al., 2008; Beckman, 2011). To improve growth and growth efficiency in aquaculture, an advanced understanding of the physiological mechanisms that regulate growth in fishes are needed. The growth hormone/insulin-like growth factor (GH/IGF) in the endocrine axis regulates growth in all vertebrates, including fishes as described by Davis et al. (2008).

Tilapia is the common name for nearly a hundred species of cichlids from the tilapinne cichlid tribe. Tilapia are mainly freshwater fish inhabiting shallow streams, ponds, rivers and lakes and less commonly found living in brackish water. The survey carried out by Oguntade et al. (2014) shows that some fish species including Tilapia are fast disappearing in Nigerian water bodies such as Brass and Nun River of Niger Delta.

In 2017, according to FAO statistics, Nile tilapia (*Oreochromis niloticus)* culture alone was ranked fourth among the most cultured in the world, in terms of both production and value with a total aquaculture production of 4.1 million tonnes (FAO, 2019). The other top four species were silver carp, grass carp, common carp and other Cyprinids (FAO, 2019). Nile tilapia represents approximately 86% of total global tilapia production (FAO, 2019). In 2017, it is anticipated that global Nile Tilapia production will reach nearly 4 million tonnes (FAO, 2017). Adult *Oreochromis niloticus (Nile* tilapia) reach up to 60 cm maximum length while *Sarotherodon melanotheron* (black chin Tilapia) has a maximum length of 28 cm (Olaosebikan and Raji, 1998) when subjected to the same environmental condition. This justifies the higher value and demand for Nile tilapia, hence higher production of other Tilapia species is needed to meet the demand for Tilapia. Black chin Tilapia on the other hand thrive well in high salinity region but are constrained by their growth and as such do not meet market value. Since IGF-I also regulate growth in fishes; the need to study its variation in the two Tilapia species arises with the aim of identifying a possible growth factor that will promote a higher production of *S. melanotheron* from saline environment to complement the production of *O. niloticus* from fresh water bodies to meet global tilapia demand. This would be achieved by detecting the genetic variations and single nucleotide polymorphisms (SNPs) in one of the main growth genes, Insulin like growth Factor-1 (IGF-I) in two Tilapia species.

MATERIALS AND METHODS

Fish sample collection and identification

Ten (10) live female tilapia fishes comprising five *Oreochromis niloticus* (ON1, ON2, ON3, ON4 and ON5) and five *Sarotherodon melanotheron* (SM1, SM2, SM3, SM4 and SM5) were obtained from Nigerian Institute for Oceanography and Marine Research (NIOMR), Badore outstation (latitude 6°25'60"N and longitude 3°51'0"E), and Oluwo market sourced from Epe river, Epe (latitude 6°35'2"N and longitude 3°59'0"E), Lagos state, Nigeria respectively. The samples of caudal fins were clipped and transferred into ten different plain 10 mL sterile tubes each containing 4 mL absolute ethanol.

Fish samples were identified from description checklist and identification keys (FAO, 1996; Froese and Pauly, 2003; Uyeno and Fujii, 1984).

DNA extraction and amplification

DNA was isolated from the caudal fin tissue of the ten sampled fish using modified chlorophenol/ isoamyl/alcohol protocol according to Sambrook and Russell (2001) on bench at biotechnology laboratory of NIOMR, Badore outstation, Nigeria. The integrity of the DNA was checked on 1% ethidium bromide stained agarose gel electrophoresis and the isolate was stored at -20°C prior PCR amplification.

The PCR amplification was run with the specific primers (IGF-I forward- 5'-CTTGGACGAGTAGGAGGCAAATG-3' and IGF-I reverse- 3'-GAAATACAAGCAAGCGATAAGAA-5') of 447 bp designed to amplify coded regions (exons) of the IGF-I gene sequences (IGF-I, GenBank accession AF033797) which was re-sequenced and used. The DNA amplification was carried out by polymerase chain reaction (PCR) in a with 20 ng of genomic DNA using Thu et al. (2017) protocol, 20 ul reactions containing 0.2 uM of each primer, 200 uM of dNTPs, 50 mM KCL, 10 mM Tris HCL (pH 8.3), 1.5 mM $MgCl_2$ and 0.5 units of Taq DNA polymerase with Eppendorf thermocycler with an amplification profile of initial denaturation at 95°C for 10 min, followed by 35 cycles with 95°C for 30s, annealing temperature at 60°C for 45s, extension at 72°C for 45s and final extension at 72°C for 5 min. The product was checked on 1.5% agarose gel electrophoresis at 70v for 1.5 h in 1x TBE buffer and the gel was stained with ethidium bromide for visualization through Fisher Scientific UV transilluminator.

DNA sequencing

Purified PCR products from the amplification of the ten Tilapia

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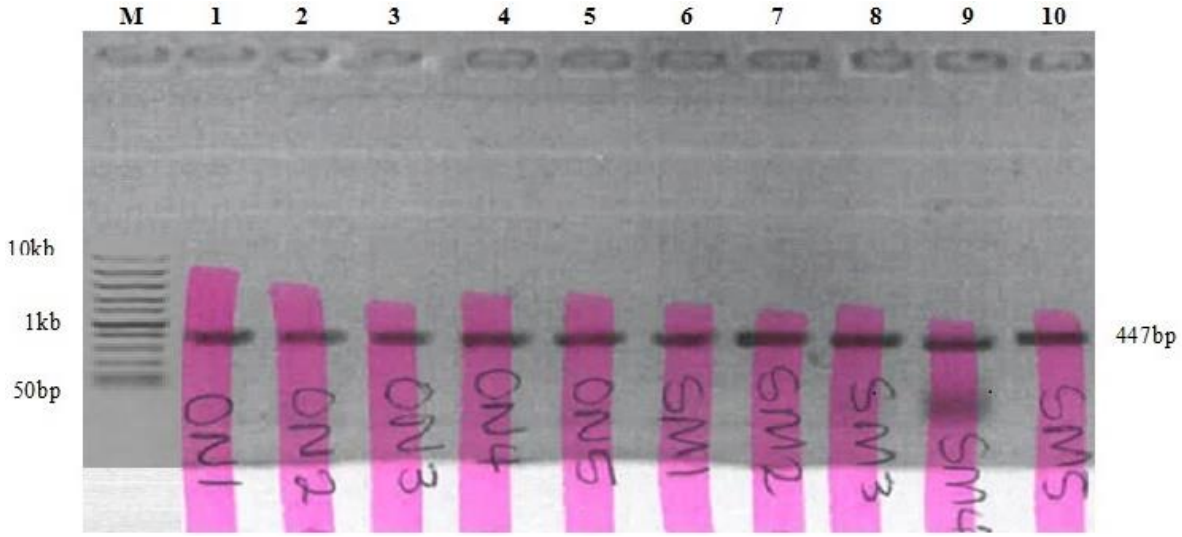

Plate 1. Picture of 1.5 % ethidium bromide stained agarose gel electrophoresis picture of the amplified DNA isolate from the ten Tilapia samples of *O. niloticus* (ON1-ON5) and *S. melanotheron (SM1-SM5).*

fishes, *O. niloticus* (5) and *S. melanotheron* (5) were bidirectional sequenced in an automatic sequencer (ABI 3500XL Genetic Analyzer).

Nucleotide sequences obtained were edited and aligned using clustal O (version1.2.4) multiple sequence alignment software, the Single nucleotide Polymorphisms (SNPs) were discovered by visual analysis and dendogram was also created while translation of the DNA sequence of each species was done using biolign alignment software (version 4.0.6.2).

RESULTS AND DISCUSSION

DNA extraction and amplification

The IGF-I 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 fragment length of the ten Tilapia fishes, *O. niloticus* (5) and *S. melanotheron* (5) as shown on Plate 1, where M is the known 50 bp- 10 kb DNA ladder.

DNA sequencing and analyses

The sequence alignment generated for the forward and reverse primers of IGF-1 of the ten tilapia fish (5 *Oreochromis niloticus* and 5 *Sarotherodon melanotheron)* were shown in Figures 1 and 2 respectively while Tables 1 and 2 show the five single nucleotide polymorphisms (SNPs) detected at the positions 181(T/C), 199(T/C), 328(C/G), 362(C/A), and 369(A/C) of the forward reaction sequence and those detected in the reverse reaction sequence at positions 18(T/A), 20(T/G), 54(C/G), 183(G/A), and 201(G/A) respectively.

The dendogram revealed lower similarities between SM1-SM5 and ON1-ON5 and higher similarities among SM1-SM5 and among ON1-ON5 (Figure 3). The lower similarity between SM and ON might imply a high genetic variation and could be due to the fact that they are different species and do not have common ancestry. This finding is on the contrary with the report of Usman et al. (2013) who obtained a high similarity coefficient of 78% between *T. guineensis* and *S. melanotheron* from the wild.

A total of 138 amino acid sequence was translated from the DNA sequence of *O. niloticus* and *S. melanotheron* as shown in Figures 4 and 5. The alignment of these sequences revealed seven (7) Variations at positions 1, 3, 4, 61, 67, 121 and 123 as shown in Figure 6.

The variations observed are as follows; position 1 R (arginine) in SM to V (valine) in ON, position 61 Q (glutamine) in SM to *(stop codon) in ON (Table 3).

This study described the use of Single Nucleotide Polymorphism (SNP) markers to validate genetic variation in a candidate growth gene (IGF-I) in *O. niloticus* and *S. melanotheron.* The DNA sequences gotten from primer used in this study was about 447 bp in agreement with the result obtained by Cuevas-Rodríguez et al. (2016).

Among the growth genes, IGF-I is said to contribute in a variety of physiological processes, such as growth, metabolism, reproduction and osmoregulation (Reinecke et al., 2005) in teleosts. Thus variation of IGF-I might be a good reason for the growth difference between *O. niloticus* and *S. melanotheron.*

It was summarized that there are indeed variations in the candidate growth gene, IGF-I of the two Tilapia species and this might be a reason for the significant difference in their growth rate. Thus improved varieties of

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CLUSTAL O(1.2.4) multiple sequence alignment

SM1_IGF-1F      ----------------AGTCTGTGTATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  44
SM3_IGF-1F      -------CTTGCAAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  53
SM4_IGF-1F      --CTTTTCTGTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  58
SM5_IGF-1F      -CGCTTTCTGTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  59
SM2_IGF-1F      CGTTTTCTTGTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  60
ON1_IGF-1F      GTTTTTTCTTTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  60
ON4_IGF-1F      --CGCCACTGATGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  58
ON2_IGF-1F      -------------ATGGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  47
ON3_IGF-1F      -CTTTTCTGGTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  59
ON5_IGF-1F      -GTTTTCTTGTTGAATGTCTGTGTAATGTAGATAAATGTGAGGGATTTTCTCTCTAAATC  59
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SM1_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  104
SM3_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  113
SM4_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  118
SM5_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  119
SM2_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  120
ON1_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  120
ON4_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  118
ON2_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  107
ON3_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  119
ON5_IGF-1F      CGTCTCCTGTTCGCTAAATCTCACTTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCG  119
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SM1_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  164
SM3_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  173
SM4_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  178
SM5_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  179
SM2_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  180
ON1_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  180
ON4_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  178
ON2_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  167
ON3_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  179
ON5_IGF-1F      GAATATTGAGATGTGACATTGCCCGCATCTCATCCTCTTTCTCCCTGTTTTTAATGACTT  179
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SM1_IGF-1F      CAAACAAGTTCATTTTCGCCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  224
SM3_IGF-1F      CAAACAAGTTCATTTTCGCCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  233
SM4_IGF-1F      CAAACAAGTTCATTTTCGCCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  238
SM5_IGF-1F      CAAACAAGTTCATTTTCGCCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  239
SM2_IGF-1F      CAAACAAGTTCATTTTCGCCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  240
ON1_IGF-1F      TAAACAAGTTCATTTTCGTCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  240
ON4_IGF-1F      TAAACAAGTTCATTTTCGTCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  238
ON2_IGF-1F      TAAACAAGTTCATTTTCGTCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  227
ON3_IGF-1F      TAAACAAGTTCATTTTCGTCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  239
ON5_IGF-1F      TAAACAAGTTCATTTTCGTCGGGCTTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCT  239
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SM1_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  284
SM3_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  293
SM4_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  298
SM5_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  299
SM2_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  300
ON1_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  300
ON4_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  298
ON2_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  287
ON3_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  299
ON5_IGF-1F      TTTTCCTTTCAGTGGCATTTATGTGATGTCTTCAAGGTAACTTACCTGATTTCCTTTGAC  299
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SM1_IGF-1F      ACTATACATTATCACCTTGATTCTTCAGTTGCTCACTATTTGCACAGAGCATCCTCGCCT 344
SM3_IGF-1F      ACTATACATTATCACCTTGATTCTTCAGTTGCTCACTATTTGCACAGAGCATCCTCGCCT 353
SM4_IGF-1F      ACTATACATTATCACCTTGATTCTTCAGTTGCTCACTATTTGCACAGAGCATCCTCGCCT 358
SM5_IGF-1F      ACTATACATTATCACCTTGATTCTTCAGTTGCTCACTATTTGCACAGAGCATCCTCGCCT 359
SM2_IGF-1F      ACTATACATTATCACCTTGATTCTTCAGTTGCTCACTATTTGCACAGAGCATCCTCGCCT 360
ON1_IGF-1F      ACTATACATTATCACCTTGATTCTTCACTTGCTCACTATTTGCACAGAGCATCCTCGCCT 360
ON4_IGF-1F      ACTATACATTATCACCTTGATTCTTCACTTGCTCACTATTTGCACAGAGCATCCTCGCCT 358
ON2_IGF-1F      ACTATACATTATCACCTTGATTCTTCACTTGCTCACTATTTGCACAGAGCATCCTCGCCT 347
ON3_IGF-1F      ACTATACATTATCACCTTGATTCTTCACTTGCTCACTATTTGCACAGAGCATCCTCGCCT 359
ON5_IGF-1F      ACTATACATTATCACCTTGATTCTTCACTTGCTCACTATTTGCACAGAGCATCCTCGCCT 359
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SM1_IGF-1F      AATTTAAACAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCA-------- 396
SM3_IGF-1F      AATTTAAACAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCAACACC--- 410
SM4_IGF-1F      AATTTAAACAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTGTATTTCA--------- 409
SM5_IGF-1F      AATTTAAACAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTGTATTTCA--------- 410
SM2_IGF-1F      AATTTAAACAGAAACAATAAAAGGGGATTCTTATCGCTTGCTTTGTATTTCAGCA----- 415
ON1_IGF-1F      ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGCTTTGTATTTCA-------- 412
ON4_IGF-1F      ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCACCGCTTTT 418
ON2_IGF-1F      ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCA-------- 399
ON3_IGF-1F      ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCA-------- 411
ON5_IGF-1F      ACTTTAAAAAGAAACAATAAAAGGGGATTCTTATCGCTTGTTTTGTATTTCA-------- 411
                * ****** ******************************** **    **

SM1_IGF-1F      ------------------------------------------------------------ 396
SM3_IGF-1F      ------------------------------------------------------------ 410
SM4_IGF-1F      ------------------------------------------------------------ 409
SM5_IGF-1F      ------------------------------------------------------------ 410
SM2_IGF-1F      ------------------------------------------------------------ 415
ON1_IGF-1F      ------------------------------------------------------------ 412
ON4_IGF-1F      TTTCGACTGCATTCATGTGGATTTATTTATATTTGCCTCTCTACTCATGCAAGCAAGA   476
ON2_IGF-1F      ------------------------------------------------------------ 399
ON3_IGF-1F      ------------------------------------------------------------ 411
ON5_IGF-1F      ------------------------------------------------------------ 411
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Figure 1. Alignment of the forward reaction of the IGF-1 of the ten tilapia fish (5 *O. niloticus* and 5 *S. melanotheron)* . *Mean similarities among the sequence bases of the ten fishes.

Table 1. SNPs detected between the forward nucleotide sequence reaction of the ten Tilapia fishes ***(****Oreochromis niloticus* and *Sarotherodon melanotheron).*

SNP position	ON	SM
181	T	C
199	T	C
328	C	G
362	C	A
369	A	C

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CLUSTAL O(1.2.4) multiple sequence alignment

SM2-IGF-1R      -----TATTGTTTTGTTTATTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAACTGAAGA 55
SM1-IGF-1R      --TTTTTGTTCCTGTTTTATTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAACTGAAGA 58
SM5-IGF-1R      ----TATTGTTTTGTTTTATTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAACTGAAGA 56
SM3-IGF-1R      ---TTATTCTTTCTGTTTATTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAACTGAAGA 57
SM4-IGF-1R      ---TTTTGGTTTCTGTTTATTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAACTGAAGA 57
ON4-IGF-1R      -TTATTGTTTCTTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGA 59
ON3-IGF-1R      TTTTGGTTTTCTTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGA 60
ON1-IGF-1R      ---TTTTTCTTCTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGA 57
ON2-IGF-1R      --TATTGGTTTCTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGA 58
ON5-IGF-1R      -TTATTGGTTTCTTTTTAAGTAGGCGAGGATGCTCTGTGCAAATAGTGAGCAAGTGAAGA 59
                         ** * ********************************** ******

SM2-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 115
SM1-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 118
SM5-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 116
SM3-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 117
SM4-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 117
ON4-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 119
ON3-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 120
ON1-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 117
ON2-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 118
ON5-IGF-1R      ATCAAGGTGATAATGTATAGTGTCAAAGGAAATCAGGTAAGTTACCTTGAAGACATCACA 119
                ************************************************************

SM2-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 175
SM1-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 178
SM5-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 176
SM3-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 177
SM4-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 177
ON4-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 179
ON3-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 180
ON1-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 177
ON2-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 178
ON5-IGF-1R      TAAATGCCACTGAAAGGAAAAAGCGCTAGACATCCCCACGGGTCTCCACAAGACAAAGCC 179
                ************************************************************

SM2-IGF-1R      CGGCGAAAATGAACTTGTTTGAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 235
SM1-IGF-1R      CGGCGAAAATGAACTTGTTTGAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 238
SM5-IGF-1R      CGGCGAAAATGAACTTGTTTGAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 236
SM3-IGF-1R      CGGCGAAAATGAACTTGTTTGAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 237
SM4-IGF-1R      CGGCGAAAATGAACTTGTTTGAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 237
ON4-IGF-1R      CGACGAAAATGAACTTGTTTAAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 239
ON3-IGF-1R      CGACGAAAATGAACTTGTTTAAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 240
ON1-IGF-1R      CGACGAAAATGAACTTGTTTAAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 237
ON2-IGF-1R      CGACGAAAATGAACTTGTTTAAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 238
ON5-IGF-1R      CGACGAAAATGAACTTGTTTAAAGTCATTAAAAACAGGGAGAAAGAGGATGAGATGCGGG 239
                ** ***************** ************************************

SM2-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 295
SM1-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 298
SM5-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 296
SM3-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 297
SM4-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 297
ON4-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 299
ON3-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 300
ON1-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 297
ON2-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 298
ON5-IGF-1R      CAATGTCACATCTCAATATTCCGACTTTGTTCCATTGCGCAGGCTCGTTTTGGAGAAGTG 299
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SM2-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 355
SM1-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 358
SM5-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 356
SM3-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 357
SM4-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 357
ON4-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 359
ON3-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 360
ON1-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 357
ON2-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 358
ON5-IGF-1R      AGATTTAGCGAACAGGAGACGGATTTAGAGAGAAAATCCCTCACATTTATCTACATTACA 359
                ************************************************************

SM2-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTTCGTCCAAGA    412
SM1-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-    414
SM5-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTATTCGTCCAAGA-    412
SM3-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-    413
SM4-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-    413
ON4-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTATTTCGTCCAAGA    416
ON3-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-    416
ON1-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-    413
ON2-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTATTCGTCCAAGA-    414
ON5-IGF-1R      CAGACATTTTCAACAGGAAACAGCTGGGGCAGCATTTGCCTCCTCTTCGTCCAAGA-    415
                ****************************************** **    * *
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Figure 2. Alignment of the Reverse Reaction of the IGF-I of the ten tilapia fish (5 *Oreochromis niloticus* and 5 *Sarotherodon melanotheron).* *Mean similarities among the sequence bases of the ten fishes.

Table 2. SNPs detected between the reverse nucleotide sequence reaction of the two Tilapia species *(O. niloticus and S. melanotheron).*

SNP position	SM	ON
81	T	A
20	T	G
54	C	G
183	G	A
201	G	A

Table 3. Variations detected between the amino acid sequence of the two Tilapia species *(O. niloticus and S. melanotheron).*

Sequence position	SM	ON
1	R	V
3	L	S
4	V	F
61	Q	*
67	P	S
121	N	T
123	N	K

*Mean stop codon.

S. melanotheron with bigger sizes might be achievable. Also, regions of higher salinity where *S. melanotheron* strive well can be encouraged to grow the improved varieties and the demand for *S. melanotheron* increases.

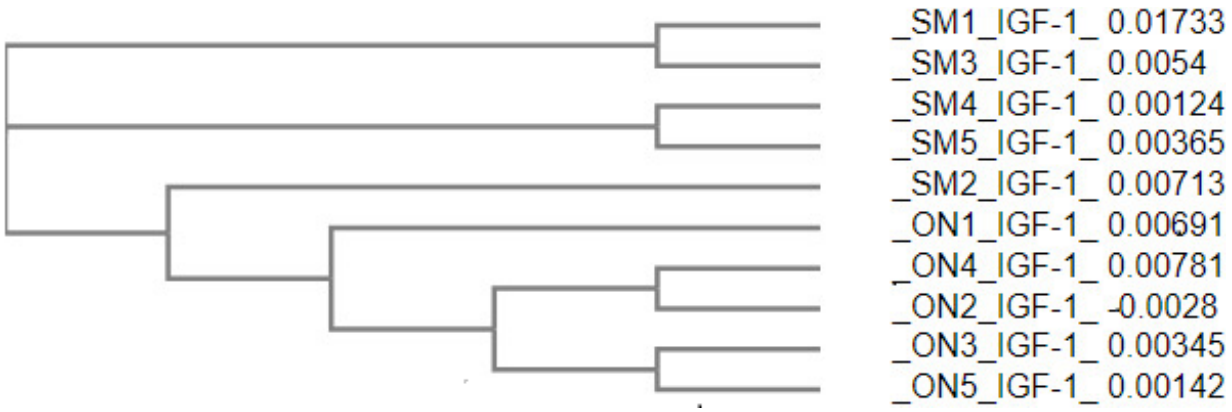

Figure 3. Dendogram (phylogenetic tree) of the DNA sequence from IGF-I for the ten Tilapia (5 *O. niloticus* and 5 *S. melanotheron*).

>(ON1_IGF-1)

```
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 *O. niloticus* (ON) to amino acid. *Mean stop codon.

```
>(SM2_IGF-1)

1     CGT TTT CTT GTT GAA TGT CTG TGT AAT GTA GAT AAA TGT GAG GGA   45
1      R   F   L   V   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   CAA ACA AGT TCA TTT TCG CCG GGC TTT GTC TTG TGG AGA CCC GTG   225
61     Q   T   S   S   F   S   P   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 GTT GCT CAC TAT TTG CAC AGA GCA TCC TCG CCT   360
106    L   D   S   S   V   A   H   Y   L   H   R   A   S   S   P   120

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

406   TAT TTC AGC   414
136    Y   F   S
```

Figure 5. Translation of DNA sequence of IGF-1 of *S. melanotheron* (SM) to amino acid. *Mean stop codon.

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    5         15         25         35         45         55
(SM2_IGF-1      RFLVECLCNV DKCEGFSL*I RLLFAKSHFS KTSLRNGTKS EY*DVTLPAS HPLSPCF**L
(ON1_IGF-1      VFSFECLCNV DKCEGFSL*I RLLFAKSHFS KTSLRNGTKS EY*DVTLPAS HPLSPCF**L

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    65         75         85         95        105        115
(SM2_IGF-1      QTSSFSPGFV LWRPVGMSSA FSFQWHLCDV FKVTYLISFD TIHYHLDSSV AHYLHRASSP
(ON1_IGF-1      *TSSFSSGFV LWRPVGMSSA FSFQWHLCDV FKVTYLISFD TIHYHLDSSL AHYLHRASSP

                ....|....| ....|...
                   125        135
(SM2_IGF-1      NLNRNNKRGF LSLALYFS
(ON1_IGF-1      TLKRNNKRGF LSLALYF.
```

Figure 6. Amino acid sequence alignment from the translation of the DNA sequence of IGF-1 of *Oreochromis niloticus* (ON) and *Sarotherodon melanotheron* (SM). * Mean stop codon.

This study serves as baseline information in selective breeding whereby the amino acids present in the IGF-I of *O. niloticus* may be fed orally to *S. melanotheron* by adding them to their feed while growing.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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