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Banat's Journal of Biotechnology
2011, II(4),

INVESTIGATION AND CHARACTERIZATION OF THE GROWTH HORMONE TYPE 1 (GH1) GENE HOMOLOGUE IN THE *SALMO TRUTTA CASPIUS*

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Abstract. *Salmo trutta caspius* growth hormone genes (GH) homologue are used as models for studies in comparative variation breeding, transgenic and growth body weight. Hence variation genetic was studied between the *salmo trutta caspius* growth hormone gene1 (GH1) and *Salmo salar*. In this study, isolation and characterization of growth hormone of gene type 1 (GH1) in the *salmo trutta caspius*, recognizing full length DNA GH1 in strain *salmo trutta caspius* on the gel electrophoresis. First, DNA genomic extracted from blood samples of adult *salmo trutta caspius* and from places north of Iran was done. The method of DNA extraction (absorption on the fiber glass (kit) were used. One pair of primers was designed towards GH1 gene homologue. DNA template concentration, annealing temperature and extension time was monitored to optimize them. PCR products have been compared between strains on the gel electrophoresis. Four fragments from nucleotide of 1 to 2541 were sequenced. The PCR products were analyzed by BLASTn. Results are shown; there were high homology within strains, *salmo trutta caspius* and *salmo salar* 96% approximately. Moreover, our discussion are shown about sequencing homologue GH1 of *salmo trutta caspius* populations possible have been common ancestor with different species *salmo trutta.*

***Key words*:** *Salmo salar*, *Salmo trutta caspius*, Growth hormone gene type 1, Sequencing, DNA

Introduction

The *Salmo trutta caspius* is one of the nine subspecies of Brown trout (*Salmo trutta*) in the world [QUILLET *et al.*, 1992], and is endemic to the Southern Caspian Sea and its tributaries [QUILLET et al., 1992]. This species is critically endangered anandaromous.

Hence, *salmo trutta caspius* has become one of the most important farmed species in aquaculture. In related to, we studied about growth hormone (GH) gene.

Growth hormone is a pituitary–secreted polypeptide hormone that in vertebrate is responsible for stimulating growth which has been shown to regulate and is involved in many other metabolic functions [PETER *et al.*, 1995].

Also, directly or indirectly, growth hormone is the main regulator of postnatal somatic growth and stimulates anabolic processes such as cell division, skeletal growth and protein synthesis [GROSS *et al.*, 1996].

From other target, the GH gene is a potential target for studies of genetic variation in connection with studies of growth traits [NILSSON *et al.*, 1995; GROSS *et al.*, 1995; GROSS *et al.*, 1996; RAYMOND *et al.*, 1995a, b].

Salmonid fishes have two type GH genes (type 1 and type 2) [AGELLON *et al.*, 1988; RENTIER *et al.*, 1989. FORBES *et al.*, 1994]. These two type genes is common ancestor that double its entire genome through tetraploidy an estimated 25 – 50 million years ago.

We studied about GH type 1 for finding polymorphism in *salmo trutta caspius* and in related to, first, were designed one pair of primers from full length of the GH1 gene's *salmo salar* (Accession nos. AY614010 [RYYNANEN *et al.*, 2004] and X61938 [MALE *et al.*, 1992]), for the sequencing.

So, we found full length polymorphism in amplification products of the GH1 gene homologue in the *salmo trutta caspius* detected polymorphism within a full length of approximately 3 kb on the gel electrophoresis.

These results showed that there were high similarities within population's *salmo trutta caspius* and *salmo salar*.

Materials and Methods:

A. Samples: The adults' *salmon* (three age years) average 500 gram, in November 2010 was collected from International Research center fishery of Tonekabon–Iran. These samples, first, were anaesthetized with MS222 and sample bloods taken from the caudal vein and transformed to

heparinized tube 1 ml for extraction DNA.

B. Extraction of DNA: Total genomic DNA was extracted from the blood samples according to absorption on fiber glass (Roche company kit) method.

C. Primers: We designed one pair of primers for amplification DNA of Growth hormone gene with the BLASTn on the NCBI Network service. This pair of primer including,

Forward Primer: 5´–ACCTACTCAACCGACCACCGCACTTTCAAG–3´

Reverse Primer: 5´–TCAATGCAGGGCAAGGCCTAATCTG–3´

These primers could amplify full length of almost 3 kb. To amplify and sequence homologous growth hormone gene from *Salmo trutta caspius*. Also we designed four primers for synthesis of fragments and sequencing it.

PCR amplification:

PCR was performed using primers (PCR cycle conditions are given below), including:

Template DNA: 1.0 µl;

Forward primer (100ng/ ml) 2.0 µl PCR Cycle condition:

Reverse primer (100ng/ ml) 2.0 µl;

dNTP mix (2.5mM each) 2.0 µl;

10X ChromTaq Assay buffer 5.0 µl;

ChromTaq enzyme (3U/ ml) 0.5 µl;

Water: 37.5 µl;

Total Reaction volume: 50.0 µl;

94°C	94°C	55°C	72°C	94°C
5 min	30 sec	30 sec	1 min	5 min
	35 cycles			

Electrophoresis: Amplified GH1 gene full length was separated by one percent agarose gel electrophoresis.

Gels were loaded at approximately 100 V until the bromophenol blue dye front reached the end of the gel. After electrophoresis, the DNA full length was visualized ethidium bromide and then was taken photos by gel DOC Bio RAD Company.

GEL Extraction and PCR purification by the kit SPIN–50 (RKT33): The kit is designed for rapid purification of plasmid DNA from standard or low–melt agarose in TAE or TBE. Features of the kit: High quality DNA and no phenol chloroform required. PCR products were gel eluted and sequenced using Gene specific forward and reverse primer. Finally, the PCR products were sent to the Choromous Geni Company–India for doing sequence.

Results

Study variations at DNA level contribute to the genetic characterization of *Salmons* we used GH of gene.

According to the annotation GH genes, these are genes linked to economic traits and polymorphism genetics which are governed by many genes, following to the sequences of the *salmon* GH1 gene were published in the BLASTn on the National centre for biotechnology information (NCBI) network service, was designed a fragment of almost 3kb.

Hence, genomic DNA was extracted from blood samples *salmo trutta caspius* *(Figure 1).*

Figure 1. Genomic DNA was extracted from blood sample *Salmo trutta caspius* was loaded on 1% agarose gel.

PCR amplification gel photo: According to reported sequences about GH1 genes at NCBI network, we have expected full length almost 3.5 kb from PCR products but are shown approximately 3 kb from full length *salmo trutta caspius* *(Figure 2).*

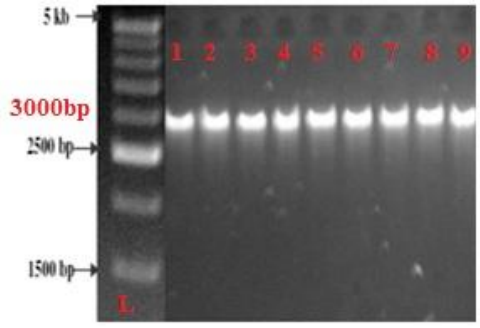

Figure 2. PCR amplification of Growth hormone gene (~ 3 kb) *Salmo trutta caspius* from samples 1 to 9. PCR Products were loaded on 1% agarose gel. L: 500bp DNA ladder.

Sequencing: For doing sequence from PCR products, we designed gene

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specific forward and reverse primers for synthesis of fragments GH1 gene. In related to were sequenced four fragments (*Figure 3*) and other PCR products were failed.

These fragments were named: *SS1FP–SS1RP–SS2FP–SS2RP*.That including:

SS1FP
CAAAAAATCATCCCTTGCGCAATTAAGAGTAAAAATGGGACAAGGTAAGCCTGCTTTTTC
TGTCTATTTCTTTTTTTAGTGGGAAGTCAGTGTACCATTTAGTACAGTTTAACTTACACA
TTTAATCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAACAAGTATTCTACTACTA
TGAGGTTATAATCTATTGACACAGAACCACCTGCTTTAACAACCTAACTATGTGATCTAT
AACATTTACATTTGAGTCATTTAGCAGACACTCTTATCCAAAGCGACTTACAGGAGCCAT
TAGGGTTAAGTGCCTTGCTCAAGGGCACATCGACAGATTTCTCACCTAGTCAGCTCAGGG
ATTGAAACCGGTAACCTTTCAATTACTTACCCAACGCTCTTAACCGCTGGGCTATTGGTG
TACAATGGCTGAGAATATCTAACTAATGTATCTCACCATAATTCGACTTACTCGTTTTAT
ACATTTCTTATTTTATTTAATCTCTCTTTTAGTGTTTCTGCTGATGCCAGTCTTACTGGT
CAGTTGTTTTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTTCAACATCGCGGT
GAACCGGGTGCAACATCTCCACCTAATGGCTCAGAAGATGTTCAATGACTTTGTAAGACA
GCTTTTGAATCTTCTTTTGACATATCAAATAATGTATTAATGATTGTTCTTCTTCTTGTA
GACAGTATCCTCTTTACACAACCCCTCGCGGCTAAAAAAAAAACAACAGAAAATCTCTCTC
CCTTTCTTTGTGATTTTGTGCAGGAAGGCACCCTGTTGCCTGATGAACGCAGACAGCTGA
AACAAGATATTCCTGCTGGAACTTTCTGTTACCTCTGACTCTATCGTGAGCCCAATCGAA
CAGCTGAGACTCAGAGAGAGTCAGTAGGTAACCTTGCCTGGAAACCATTTCACGCTCTGT
TTACGCCCCCTCT

SS1RP
CCAACCCGCCAAATTTAAGGGGTTTTAAGGGCGTCCATGCGTATTGTCTCAGCCAGGTTA
CTTACTGAACTCTTCTGAGTCTCAAGCTTGTCGATTGGGCTCACGATGGAGTCAGAGTTA
CAGAAGTCCAGCAGGAATATCTTGTTCAGCTGTCTGCGTTCATCAGGCAACAGGGTGCCT
TCCTGCACAAAATCACAAAGAAGGGAGAGAGATTTTCTGTTGTTTTTTTTTAGCCGCGAG
GGTTGTGTAAAGAGGATACTGTCTACAAAAAAAAAAAACAATCATTAATACATTATTTGAT
ATGTCAAAAAAAAATTCAAAAGCTGTCTTACAAAGTCATTGAACATCTTCTGAGCCATTA
GGTGGAGATGTTGCACCCGGTTCACCGCGATGTTGAAAAGCCGTTGGTTTTCCATCGCTG
CCCCTTGGCTCAAAAAACAACTGACCAGTAAGACTGGCATCAGCAAAAACACTAAAAGAG
AGATTAAATAAAATAAGAAATGTATAAAACGAGTAAGTCGAATTATGGTGAGATACATTA
GTTAGATATTCTCAGCCATTGTACACCAATAGCCCAGCGGTTAAAAGCGTTGGGTAAGTA
ATTGAAAGGTTACCGGTTTCAATCCCTGAGCTGACTAGGTGAGAAATCTGTCGATGTGCC
CTTGAGCAAGGCACTTAACCCTAATGGCTCCTGTAAGTCGCTTTGGATAAGAGTGTCTGC
TAAATGACTCAAATGTAAATTGTTATAGATCACATAGTTAGGTTGTTAAAGCAAGTGGTT
CTGTGTCAATAGATTATAACCTCATAGTAGTAGAATACTTGTTCACCTTTTCCTCTGCCG
TGTGGGCCCTGCCTCAGTGAATTAAATGTGTAAGTTAACTGTACTAATTGGTTACCTTGA
ACTCCACTAAAAAAGAATAGGAACGAAAAGCGGCTACTGTTCCATTTTTTAGCTTCTCAA
TAGC

SS2FP
ACCGAAACTTATTTTCCATAATCTGTGGTTTCTCTACACACACAGGTCCTGAAGCTGCTC
CATATCTCTTTCCGTCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGACCATCTCC
AACAGCCTAATGGTCAGAAACTCCAACCAGATCTCTGAGAAGCTCAGCGACCTCAAAGTG
GGCATCAACCTGCTCATCAAGGTAAAGAAAGGAGGGAGAACAATGACCATTTGTGGTGCC
ACACTTTGTGCACTGTAAACCCCAAGGCATTTTTAACTCAAATACTTCTAGTAAGTTGAA
GTTG

SS2RP
TCATTGCGGGTTAAGTGCACAAAGTGTGGCACCACAAATGGTCATTGTTCTCCCTCCTTT
CTTTACCTTGATGAGCAGGTTGATGCCCACTTTGAGGTCGCTGAGCTTCTCAGAGATCTG
GTTGGAGTTTCTGACCATTAGGCTGTTGGAGATGGTCAGGGTCTGGCTAGGGTACTCCCA
GGATTCAATCAGACGGAAAGAGATATGGAGCAGCTTCAGGACCTGTGTGTGTAGAGAAAC
CACAGATTATTGGAAAATGAAGTTGCGTTGTATTGTACAGCTTGAGATTCCCTATTTACA

Figure 3. *Salmo trutta caspius* DNA sequencing from 1 to 2541 nucleotides. Major fragment was sequenced to four segments. Segments including A–T–C richness regions.

Discussion

In recent decade, have been more studies about economic traits and polymorphism genetics in *salmonids* in world.

The *Salmo trutta caspius* is one of the unique fishes in the Caspian Sea.

These fishes prefer to live in south, west and southwest of the Caspian Sea that which is the related to deeper [Berg, 1962].

These fishes has well growth, body weight and specific growth rate were significantly is high, has been reported until

maximum weight 50 kg in Russia and 25 kg | in Iran [Uzbekova. *et al.*,2000].

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Query 6 AATCATCCCTTGCGCAATTAAGAGTAAAAATGGGACAAGGTAAGCCTGCTTTTTCTGTCT 65
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Sbjct 611  AATCAT–CCTTG–GCAATTAAGAGTAAAAATGGGACAAGGTAAGCCTGCTTTTTCTGTCT 668
Query 66  ATTTCttttttAGTGGGAAGTCAGTGTACCATTTAGTACAGTTTAACTTACACATTTAA 125
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Sbjct 669  ATTTCTTTTTTCAGTGGGAAGTCAGTGTACCATTTAGTACAATTTAACTTACACATTTAA 728
Query 126  TCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAACAAGTATTCTACTACTATGAGG 185
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Sbjct 729 TCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAACAAGTATTCTACTACTATGAGG 788
Query 186 TTAT–AATCTATTGACACAGAACCACCTGCTTTAACAACCTAACTATGTGATCTATAACA 244
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Sbjct 789 TTATAAATCTATTGACACAGAACCACCTGCTTTAACAACCTAACTATGTGATCTATAACA 848
Query 245 TTTACATTTGAGTCATTTAGCAGACACTCTTATCCAAAGCGACTTACAGGAGCCATTAGG 304
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Sbjct 849 TTTACATTTGAGTCGTTTAGCAGACGCTCTTATCCAGAGCGACTTACAGGAGCAATTAGG 908
Query 305 GTTAAGTGCCTTGCTCAAGGGCACATCGACAGATTTCTCACCTAGTCAGCTCAGGGATTG 364
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Sbjct 909 GTTAAGTGCCTTGCTCAAGGGCACGTCGACAGATTTCTCACCTAGTCAGCTCAGGGATTG 968
Query 365 AAACCGGTAACCTTTCAATTACTTACCCAACGCTCTTAACCGCTGGGCTATTGGTGTACA 424
||||| |||||||||||||||||||||||||||| |||||||||| |
Sbjct 969 AAACCAGTAACCTTTCAATTACTTACCCAACGCTCTTAACCGCTAGGCTATTGGTGTTCG 1028
Query 425 ATGGCTGAGAATATCTAACTAATGTATCTCACCATAATTCGACTTACTCGTTTTATACAT 484
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Sbjct 1029 ATGGCTGAGAATATCTAACTAATGTATCTCACCATAATTCGACTTACTCGTTTTATACAT 1088
Query 485 TTCTTATTTTATTTAATCTCTCTTTTAGTGTTTCTGCTGATGCCAGTCTTACTGGTCAGT 544
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Sbjct 1089 TTGTTA——T–TTT–CTCTTTCTTTTAGTGTTTCTGCTGATGCCAGTCTTACTGGTCAGT 1143
Query 545 TGTTTTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTTCAACATCGCGGTGAAC 604
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Sbjct 1144 TGTTTTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTTCAACATCGCGGTCAAC 1203
Query 605 CGGGTGCAACATCTCCACCTAATGGCTCAGAAGATGTTCAATGACTTTGTAAGACAGCTT 664
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Sbjct 1204 CGGGTGCAACATCTCCACCTAATGGCTCAGAAGATGTTCAATGACTTTGTAAGACAGCTT 1263
Query 665 TTGAATCTTCTTTTTGACATATCAAATAATGTATTAATGATTGTTCTTCTTCTTGTAGACA 724
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Sbjct 1264 TTGAATCTTCTTTTGACATATCAAATAGTGTATCAATGATTGTTCTTCTTCTTGTAGACA 1323
Query 725 GTATCCTCTTTACACAACCCCTCGCGGCTaaaaaaaaaCAACAGAAAA–TCTCTCTCCCT 783
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Sbjct 1324 GTGTCCTCTTTACACAACCC–TCGTGGCAA————CAACAAAAAAATCTCTCTCCCT 1374
Query 784 TTCTTTGTGATTTTGTGCAGGAAGGCACCCTGTTGCCTGATGAACGCAGACAGCTGAAAC 843
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Sbjct 1375 T–CTTTGTGATTTTGTGCAGGAAGGTACCCTGTTGCCTGATGAACGCAGACAGCTGAA–C 1432
Query 844  AAGATATTCCTGCTGGAACTTTCTGTTACCTCTGACTCTATCGTGAGCCCAATCGAACA– 902
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Sbjct 1433 AAGATATTCCTGCTGGA–CTT–CTGTAAC–TCTGACTCCATCGTGAGCCCAATCGA–CAA 1488
Query 903 GCT–GAGACTCAGAGAGAGT–CAGTAGGTAACCT 934
||| ||||||||| ||||| ||||| ||||||
Sbjct 1489 GCTTGAGACTCAGA–AGAGTTCAGTAAGTAACCT 1521
Identities = 870/947 (92%), Gaps = 40/947 (4%)
Query 994 GGTTTT–AAGGGCGT–CCATGCGT–ATTGTCTCAGCCAGGTTACTTACTGAACTCTTCTG 1050
||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1558 GGTTTTAAAGGGCATAACATGCATAATTGTCTCAACCAGGTTACTTACTGAACTCTTCTG 1499
Query 1051 AGTCTCAAGCTTGTCGATTGGGCTCACGATGGAGTCAGAGTTACAGAAGTCCAGCAGGAA 1110
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Sbjct 1498 AGTCTCAAGCTTGTCGATTGGGCTCACGATGGAGTCAGAGTTACAGAAGTCCAGCAGGAA 1439
Query 1111 TATCTTGTTCAGCTGTCTGCGTTCATCAGGCAACAGGGTGCCTTCCTGCACAAAATCACA 1170
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Sbjct 1438 TATCTTGTTCAGCTGTCTGCGTTCATCAGGCAACAGGGTACCTTCCTGCACAAAATCACA 1379
Query 1171 AAGAAGGGAGAGAGATTTTCT–GTTGttttttttAGCCGCGAGGGTTGTGTAAAGAGGA 1229
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Sbjct 1378 AAGAAGGGAGAGAGATTTTTTTGTTGTT————GCCACGAGGGTTGTGTAAAGAGGA 1327
Query 1230 TACTGTCTACaaaaaaaaaaCAATCATTAATACATTATTTGATATGTCaaaaaaaaTT 1289
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Sbjct 1326 CACTGTCTACAAGAAGAAGAACAATCATTGATACACTATTTGATATGTCAAAAGAAGATT 1267
Query 1290 CAAAAGCTGTCTTACAAAGTCATTGAACATCTTCTGAGCCATTAGGTGGAGATGTTGCAC 1349
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Sbjct 1266 CAAAAGCTGTCTTACAAAGTCATTGAACATCTTCTGAGCCATTAGGTGGAGATGTTGCAC 1207
Query 1350 CCGGTTCACCGCGATGTTGAAAAGCCGTTGGTTTTCCATCGCTGCCCCTTGGCTCAAAAA 1409
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Sbjct 1206 CCGGTTGACCGCGATGTTGAAGAGCCGTTGGTTTTCCATCGCTGCCCCTTGGCTCAGAAA 1147
Query 1410 ACAACTGACCAGTAAGACTGGCATCAGCAAAAACACTAAAAGAGAGATTAAATAAAATAA 1469
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Sbjct  1146  ACAACTGACCAGTAAGACTGGCATCAGCAGAAACACTAAAAGAAAGAG–AAA–A—–TAA 1092
Query 1470 GAAATGTATAAAACGAGTAAGTCGAATTATGGTGAGATACATTAGTTAGATATTCTCAGC 1529
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Sbjct 1091 CAAATGTATAAAACGAGTAAGTCGAATTATGGTGAGATACATTAGTTAGATATTCTCAGC 1032
Query 1530 CATTGTACACCAATAGCCCAGCGGTTAAAAGCGTTGGGTAAGTAATTGAAAGGTTACCGG 1589
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Sbjct 1031 CATCGAACACCAATAGCCTAGCGGTTAAGAGCGTTGGGTAAGTAATTGAAAGGTTACTGG 972
Query 1590 TTTCAATCCCTGAGCTGACTAGGTGAGAAATCTGTCGATGTGCCCTTGAGCAAGGCACTT 1649
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Sbjct 971 TTTCAATCCCTGAGCTGACTAGGTGAGAAATCTGTCGACGTGCCCTTGAGCAAGGCACTT 912
Query 1650 AACCCTAATGGCTCCTGTAAGTCGCTTTGGATAAGAGTGTCTGCTAAATGACTCAAATGT 1709
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Sbjct 911 AACCCTAATTGCTCCTGTAAGTCGCTCTGGATAAGAGCGTCTGCTAAACGACTCAAATGT 852
Query 1710 AAATTGTTATAGATCACATAGTTAGGTTGTTAAAGCAAGTGGTTCTGTGTCAATAGATT–1768
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Sbjct 851 AAAT–GTTATAGATCACATAGTTAGGTTGTTAAAGCAGGTGGTTCTGTGTCAATAGATTT 793
Query 1769 ATAACCTCATAGTAGTAGAATACTTGTTCACCTTTTCCTCTGCCGTGTGGGCCC–TGCCT 1827
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Sbjct 792 ATAACCTCATAGTAGTAGAATACTTGTTCAC–TTTTC–TCTGCCGTGTTGGCCCCTGCCT 735
Query 1828 CAGTGAATTAAATGTGTAAGTTAACT–GTACTAATTGGTTAC–CTTGAACT–CC–ACT–A 1882
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Sbjct 734 CAGTGA–TTAAATGTGTAAGTTAAATTGTACTAAATGGT–ACACT–GA–CTTCCCACTGA 679
Query 1883 AAAAAGAA–TAGGA–ACGAAAA–GC–GGCT–AC–T–GTTCCATTTTT 1922
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Sbjct 678 AAAAAGAAATAG–ACA–GAAAAAGCAGGCTTACCTTGTCCCATTTTT 634
Identities=265/265 (100%), Gaps = 0/265 (0%)
Query 1973 ACACACACAGGTCCTGAAGCTGCTCCATATCTCTTTCCGTCTGATTGAATCCTGGGAGTA 2032
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Sbjct 2324 ACACACACAGGTCCTGAAGCTGCTCCATATCTCTTTCCGTCTGATTGAATCCTGGGAGTA 2383
Query 2033 CCCTAGCCAGACCCTGACCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGATCTC 2092
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Sbjct 2384 CCCTAGCCAGACCCTGACCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGATCTC 2443
Query 2093 TGAGAAGCTCAGCGACCTCAAAGTGGGCATCAACCTGCTCATCAAGGTAAAGAAAGGAGG 2152
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Sbjct 2444 TGAGAAGCTCAGCGACCTCAAAGTGGGCATCAACCTGCTCATCAAGGTAAAGAAAGGAGG 2503
Query 2153 GAGAACAATGACCATTTGTGGTGCCACACTTTGTGCACTGTAAACCCCAAGGCATTTTTA 2212
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Sbjct 2504 GAGAACAATGACCATTTGTGGTGCCACACTTTGTGCACTGTAAACCCCAAGGCATTTTTA 2563
Query 2213 ACTCAAATACTTCTAGTAAGTTGAA 2237
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Sbjct 2564 ACTCAAATACTTCTAGTAAGTTGAA 2588
Identities=296/311 (95%), Gaps = 15/311 (5%)
Query 2245 TTGCGGG–TTA–AGTGCACAAAGTGTGGCACCACAAATGGTCATTGTTCTCCCTCCTTTC 2302
||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2553 TTG–GGGTTTACAGTGCACAAAGTGTGGCACCACAAATGGTCATTGTTCTCCCTCCTTTC 2495
Query 2303 TTTACCTTGATGAGCAGGTTGATGCCCACTTTGAGGTCGCTGAGCTTCTCAGAGATCTGG 2362
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Sbjct 2494 TTTACCTTGATGAGCAGGTTGATGCCCACTTTGAGGTCGCTGAGCTTCTCAGAGATCTGG 2435
Query 2363 TTGGAGTTTCTGACCATTAGGCTGTTGGAGATGGTCAGGGTCTGGCTAGGGTACTCCCAG 2422
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Sbjct 2434 TTGGAGTTTCTGACCATTAGGCTGTTGGAGATGGTCAGGGTCTGGCTAGGGTACTCCCAG 2375
Query 2423 GATTCAATCAGACGGAAAGAGATATGGAGCAGCTTCAGGACCTGTGTGTGT———AGA 2476
||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2374 GATTCAATCAGACGGAAAGAGATATGGAGCAGCTTCAGGACCTGTGTGTGTGTGTGTAGA 2315
Query 2477–G–A–A–A—CCACAGATTATTGGAAAATGAAGTTGCGTTGTATTGTACAGCTTGAGATT 2530
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Sbjct 2314 TGTAGAGAAACCACAGATTATTGGAAAATGAAGTTGCGTTGTATTGTACAGCTTGAGATT 2255
Query 2531 CCCTATTTACA 2541
|||||||||
Subject 2254 CCCTATTTACA 2244
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Figure 4. GH1 gene *salmo trutta caspius* fragments aligned with the *salmo salar* by NCBI program. Fragments including the four primers as started from first of length.

Growth hormone (GH) plays a very important role in many regulatory, metabolic and developmental processes in *salmonids*. GH in *salmonids* is represented by duplicated, non–allelic isoforms designated as GH1 and GH2.

At previous studies, used GH1 gene for the analysis of the genetic diversity among differences of species *salmo salar*.

Hence this gene is important for the understanding of the evolutionary process occurring in *salmo trutta caspius* after of

biogeographic separation and its relevance for the design and implementation of effective control measures.

Primers and DNA sequencing

GH1 gene has full length almost 4700 bp from seven different salmon populations (GenBank Accession nos AY614002–AY614008) were aligned [RYYNANEN, *et al*.,2004 MALE *et al*.,1992].

In *salmo trutta caspius* were designed a pair of primers for amplify of full length, polymorphism was assessed at 9 samples and was observed on the gel a fragment of almost 3kb, were purified PCR products and designed primers for the sequencing.

These primers is expected to amplify four fragments almost, 940bp, 900bp, 310bp and 300bp (positions 1 to 2540).

In related to, encompassing the fragments, positions of richness A–T–C, these characters are indicate unique at *salmo trutta caspius* which almost like nucleotides situation in the *salmo salar*.

This resulted also in higher frequency of the nucleotides AA, AT and AC compared to the lower frequency of the nucleotides GG and GC.

These are fragments aligned with other sequences, especially *salmo salar* (accession no. X61938.1), these results are shown, there is high homology 95%, in first fragment (900bp F), 92%, in second fragment (900bp R), 100% first fragment (300bp F) and 95% second fragment (300bp R) (*Figure 4*). Identities=880/934 **(94%)**, Gaps=29/934 (3%).

Conclusion:

We were sequenced a segment of 2541 bp, from first of the gene GH1.

At this study describes variation at *salmo trutta caspius* GH1 gene detected by NCBI tree aligning (*figure 5*)

Results are shown, *salmo trutta caspius* possible has been ancestral with *salmo salar*, *salmo trutta* (has been Bolded) and homology with *onchorhyncus mykiss* and *chun salmon*.

Also, we assumed this gene is candidate for evolutionary polymorphism in *salmons.*

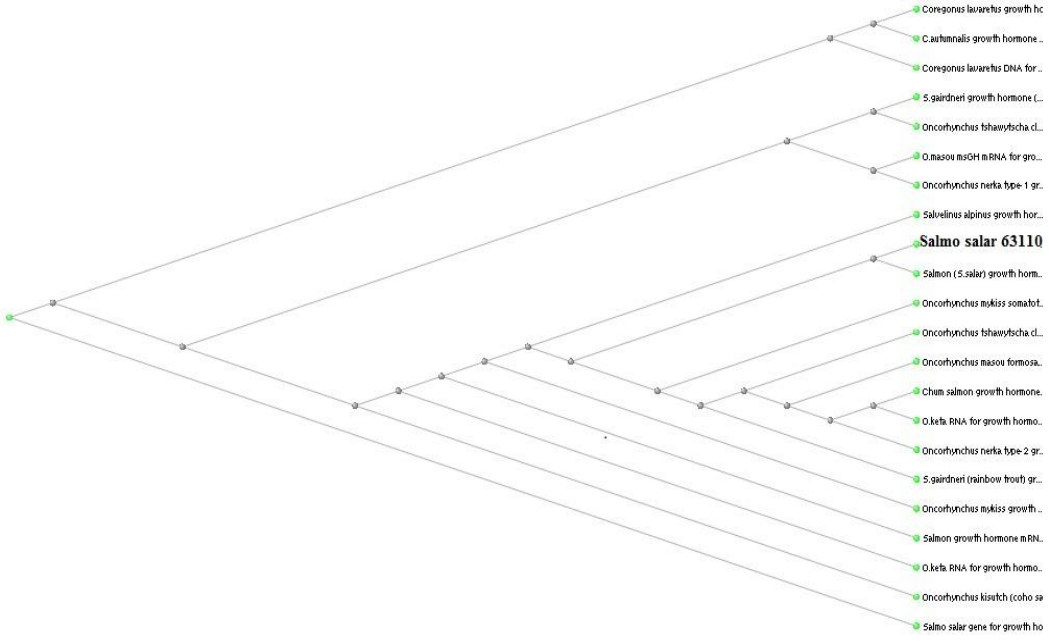

Figure 5. The feature of tree alignment by NCBI program in *salmo trutta caspius* and different population's *salmon*, results are shown; *salmo trutta caspius* and other *salmons*. Scores denote conserved direct row.

Acknowledgments

This work had financially supported by the research council of Islamic Azad University Tonekabon branch.

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Received: June 21, 2011
Accepted: August 23, 2011