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Variation in growth hormone (GH) of gene in exon sequence in three *salmon* types

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ABSTRACT

We aimed to identify polymorphisms in the coding regions of the *Salmo trutta caspius* (*s. t. caspius*) growth hormone gene for comparison of the rate homology between sequences cited regards *salmonids*, because the variation between sequences of exons is very important at finally can change in the rate of expression of gene growth hormone, however this gene identified by marker genetics in *salmonids*, and in those of *Salmo salar* (*s. salar)* and *salmo trutta (s. trutta*). This single copy nuclear gene contains six exons, that the length of the exon fragments almost 1900 base pairs between nucleotide 35 and 1907. In *s. t. caspius*. Selective PCR reaction, and sequenced the products, that including fragments of from first to end of the GH gene. We also describe a novel polymorphism of the six exons fragment, these fragments indicated for studies regards evolution of fish GH genes, phylogeny of fishes, and genetic selection. The segments of GH exon were analyzed by DNAMAN program genetics. The results are shown, there were almost 90%, homology between the first three exons of *S. t. caspius* and *S. salar* accession number (AY614002.1) Regards other *Salmons*; *S. salar* (Accession number, M21573.1) and *Rainbow trout* there weren't any homology, exon fragments second, third, fourth, fifth and sixth, the homology were high (almost 90-95 %), however between fragments of *salmons,* the rate of homology were high but the length of fragments between *salmons* were different. The length of fragments in *s. salar* more than other *salmons* including *R. trout* and *S. t. caspius*.

Keywords: *Salmo trutta caspius*, Growth hormone gene. *Salmo salar*, *Rainbow trout* (*Salmo trutta*), exon

INTRODUCTION

A fish species which has been intensively studied with allozyme (Ferguson *et al*., 1989), mitochondrial (Breatchez *et al.,* 1992; Ovenden *et al.,* 1993), and microsatellite markers (Estoup *et al.,* 1993). We studied regards Growth hormone gene. The Growth rate of fish is an important factor in aquaculture (Agelon *et al*., 1988; Gross *et al.,* 1995; Gross *et al*., 1996). Growth hormone (GH) is involved in linear growth, food conversion and appetite in fish stimulates somatic growth in vertebrates including teleosts. The use of GH for enhancing growth rate of fishes in aquaculture prompted the cloning and characterization of the GH genes from different fish species. These have been found to be more variable than mammalian GH genes. For example, carp species such as common carp (Chiou *et al.,* 1990), grass carp (Ho., 1991; Zhu *et al.,* 1992), and silver carp (Hong and Schartl, 1993), and catfish (Tang *et al.,* 1993), contain five exons, as found in mammals and birds, whereas, the *Salmonids*, *Rainbow trout* (*R.trout*) (Agelon *et al.,* 1993; ,Gross *et al.,* 1995; Gross *et al.,* 1996), *Atlantic salmon* (Johanson *et al*., 1989;Male *et al.,* 1992), *chinook salmon* (Du *et al.,* 1993), *sockeye salmon* (Devlin *et al.,* 1993), *tilapia* (Breatchez *et al.,* 1992), *flounder* (Tanaka *et al*., 1995), *yellowtail* (Ohkubo *et al.,* 1996),

barramundi (Yowe and Epping, 1995), and *pufferfish* (Venkatesh *et al.,* 1997). Contain six exons, and the gene in different species *Salmonids* also of varies from 3.5 kb in length. Variation in the length of exons and introns being low. Identification of GH gene allelic variation may provide useful genetic markers for selection of fish with desirable growth trait. The *Salmo trutta caspius* species is a marine teleost that is very important in the industrial aquaculture. This species is inhabiting Rivers around the Caspian sea, especially in the west and southwest of the Caspian sea regions. We analysed here on the sequence of GH gene, the rate of single nucleotide variation, also the studies of microsatellite fragments in the exons of *Salmo trutta caspius* and compared that with sequences for *Salmo salar* and other bonyfishes available on GeneBank. The results are shown that there are high homology between sequences of exon, but the length of GH exons in the *Salmo trutta caspius* more than shorter *Salmo salar* and other bonyfishes.

MATERIALS AND METHODS

Salmo trutta caspius were obtained from Dohezar and Sardabroud-Tonekabon-Iran. Samples including 14 numbers. They were anaesthetized with MS2220 and extracted of blood from caudal vein. Blood samples (2–5 ml) were removed from the fish via caudal puncture (G.18 needle) using a heparinized syringe (as an anticoagulant for blood sampling) after using MS222, (1:10,000) as an anaesthetic to minimize stress. The genomic DNA was extracted from Bloods sample. Were used proteinase K for digestion and phenol–chloroform for extraction (Sambrook *et al*., 1989). The concentration of DNA was checked through spectrophotometrically under wave length of A260/280 nanometer. Moreover, the samples of *Salmons* are very rarely, so for getting more DNA templates were extracted DNA by the phenol-chloroform extraction method. DNA quality was checked by 0.8 percent agarose gel electrophoresis. For getting good quality DNA, those devoid of smear were used for the study of PCR polymorphism.

PCR Amplification: A 1.9 kb. fragment of the growth hormone gene that contains the six exons from first to end of the GH gene, designed three pairs of primer using the primer walking technique. These had the sequence:

Forward primer:
ACATACTCAACCGACCACCGCACTT TCAAG
Reverse primer:
GTGACAGGTCCACTCTGCTATTCA
Forward Primer:
GTAAATAGGGAATCTCAAGCTGT
Reverse Primer:
CTCAAATACTTCTAGTAAGTTGA
Forward primer:
CATCACTAATATTGACTATATCAG
Reverse primer:
CAGATTAGGCCTTGCCCTGCACTGA

PCR was carried out in a final volume of 50 µl reaction mixture containing 80-100 ng DNA, 5 µl 10X PCR assay buffer, 2 µl. 2.5 mM of each dNTP, 0.5µl. 3u/µl. Taq DNA polymerase, 2 µl.(100ng/µl) of each primer and 37.5 µl water (D.D.W). PCR reactions were carried out in a thermocycler (PTC-200, MJ Research, USA). The PCR reaction used 10 microgram PCR reactions contained: 1 µl template DNA, 2µl forward primer (100 ng/µl), 2 µl reverse primer (100 ng/µl), 2 µl dNTP mix (2.5mM each), 5 µl 10X ChromTaq Assay buffer, 0.5 µl ChromTaq enzyme (3U/µl), Water 37.5 µl, in a total volume, 50 µl. 94° of 5 min, 35 cycles of 94°C 30 Sec., 55°C 30 Sec., and 72°C 1 min. Two to ten µl of each PCR reaction were run on 0.8 % agarose gels in TAE buffer containing ethidium bromide. One µl 500bp, DNA ladder (Gibco-BRL) was used as a size standard. The PCR products were separated by electrophoresis in a TAE agarose gel containing ethidium bromide using standard protocols(19) The desired PCR product band was excised using a sterile razor blade or scalpel (band was visualized in a medium or long wavelength (e.g., ≥300 nm. UV light). The agarose slice was transferred to a 1.5 ml microcentrifuge or screw cap tube and then

purified by Gel Extraction Spin-50 (RKT33) kit, Chromous Geni-India. Quantification was by loading one µl of eluted sample in 1% agarose gel and comparing with standard molecular marker (500 bp DNA ladder). Only samples with good concentration (>50 ng/µl) were selected and subjected to sequencing. Sequence data were analyzed mostly by DNAMAN program genetics and BLAST network system. The database search of sequences for a possible match to the DNA sequence of growth hormone gene was conducted using the BLAST algorithm available at the National Center for Biotechnology Information (NCBI).

RESULTS

Identification and characterization exon fragments growth hormone gene:

The PCR amplification generated approximately a 1.9 kb. This polymorphism was confirmed by nucleotide sequencing. (35..44, 513..652, 804..920, 1064..1219, 1474..1620, 1845..1907). (Fig.1). The nucleotide sequences as well as deposited in (Gene Bank, accession number, JN241634.1) have been explained in this study.

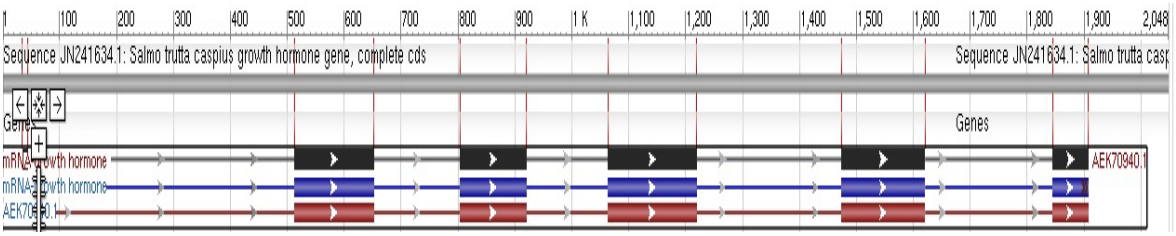

Fig. 1: Structure of graphical growth hormone gene in the *Salmo trutta caspius* (Accession number: JN241634.1). Exons are labeled, Exon1, Exon 2, Exon 3, Exon 4, Exon 5 and Exon 6. Size is indicated one base pairs (bp.). Positions and directions of exons are shown by color arrows.

Comparison of sequencing fragments exon GH gene *Salmo trutta caspius* with other Salmons: *Salmo trutta caspius* GH1 gene have six exons in the full length of GH gene. The exons have high homology with other Salmons but the length of GH shorter than Salmons GH gene. The sequences of first exons for *Salmo trutta caspius* GH gene with other species of Salmons has been depicted in. First exon has ten nucleotides in the full length of GH gene, that started from 35th base to 44th base. The comparisons of result are shown that a similarity (10 -nt consensus) between first exon of GH in the *Salmo trutta caspius* and *Salmo salar*, but regarding *Rainbow trout* there was not any nucleotides in the spanning of the first exon. (Fig. 2). There are in the Gene Bank eleven reports about full length of *Salmo salar* GH gene, we aligned sequences (Accession number: AY61402, AY61403, AY61404, AY61405, AY61406, AY61407, AY61408, AY61409, AY614010, M21573.1, and X61938.1).

```
S.s.(AY614002.1) TAAAAATGGGACAAGGTAAG    652
S.t.Caspius(E1)  .....ATGGGACAAG.....     10
S.s.(X61938.1)   TAAAAATGGGACAAGGTAAG    705
R.t.(M22731.1)   ....................      0
S.s.(M21573.1)   gAgAAAaaaatgggacaAgG    681
Consensus
```

Fig. 2: The sequences of first exon of GH gene in the *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.)

The results are shown there are high homology between sequences of Accession number from AY61402 to AY614010 and X61938.1. but with Accession number M21573.1, homology was different. In related to we selected three sequences (AY61402 and X61938.1) and M21573.1, regarding the *Rainbow trout*, there is one report about full length of mRNA of GH gene in the Gene Bank. So, we selected and were aligned with fragments of exon. Regarding to first exon, the results also

were aligned with homology tree in the DNAMAN program genetics (Fig. 3). The among of homology between *Salmo trutta caspius* and sequences of Salmons except Accession No. M21573.1 is very high, but regarding *Rainbow trout* and *Salmo salar* Accession no. M21573.1 we did not find any significant homology in this region. The start codon ATG was began from nucleotide of 35 but in *Salmo salar* (AY614002.1) from nucleotide of 638 and *Salmo salar* (X61938.1) nucleotide of 691. Regarding to *Salmo salar* (M21573.1) in this region was not observed any start codon. Regarding to Second exon, in the *Salmo trutta caspius* GH gene has 104 nucleotides.

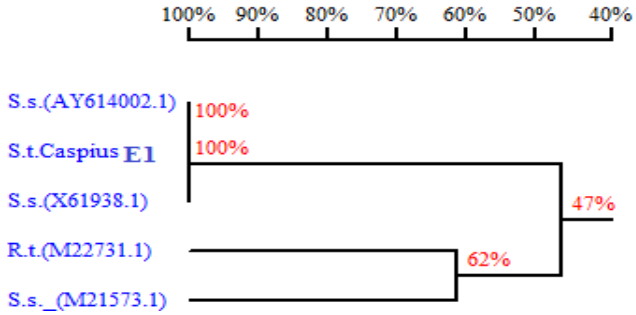

Fig. 3: Homology tree analysis of sequences of the first exon from *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.).

The results were aligned with DNAMAN program genetics (Fig. 3), we are shown that there were high homology between *Salmons* except *Salmo salar* (Acc.no. M21573.1) and *Rainbow trout* we didn't find any homology with second exon. The second exon in the *Salmo trutta caspius* GH gene is much shorter than the corresponding second exon for the Salmons. Also regarding *Rainbow trout* the results same with first exon. Approximately, there were rich of nucleotides of A-T-C repeated in the length of second exon that can be annotated mini and microsatellites regarding GH1 gene in the second exon (Fig. 4).

```
S.s.(AY614002.1) TTAGTGTTTCTGCTGATGCCAGTCTTACTGGTCAGTTGTT   1147
S.t.Caspius(E2)  ....TGTTTCTGCTGATGCCAGTCTTACTGGTCAGTTGTT    36
S.s.(X61938.1)   TTAGTGTTTCTGCTGATGCCAGTCTTACTGGTCAGTTGTT   1200
R.t.(M22731.1)   .....                                       0
S.s.(M21573.1)   TTtagtgTTtctgctgatgCcagtcTtactGgtcagTtgT   1176
Consensus
S.s.(AY614002.1) TTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTT   1187
S.t.Caspius(E2)  TTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTT     76
S.s.(X61938.1)   TTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTT   1240
R.t.(M22731.1)   .....                                       0
S.s.(M21573.1)   TTtctgagtcAaGGGgcagcgatGgAAAaCcAacGgctcT   1216
Consensus
S.s.(AY614002.1) CAACATCGCGGTCAACCGGGTGCAACATCTCCACCTAATG   1227
S.t.Caspius(E2)  CAACATCGCGGTgAACCGGGTGCAACATCTCCACCTAATG    116
S.s.(X61938.1)   CAACATCGCGGTCAACCGGGTGCAACATCTCCACCTAATG   1280
R.t.(M22731.1)   .....                                       0
S.s.(M21573.1)   tcAacatcgcGgtcAaCcGGgtgcAacatctCcaCctAct   1256
Consensus
S.s.(AY614002.1) GCTCAGAAGATGTTCAATGACTTTGTAAGACAGCTTTTGA   1267
S.t.Caspius(E2)  GCTCAGAAGATGTTCAATGACTTT.....              140
S.s.(X61938.1)   GCTCAGAAGATGTTCAATGACTTTGTAAGACAGCTTTTGA   1320
R.t.(M22731.1)   .....                                       0
S.s.(M21573.1)   GgctcagAaAatgTtcAatgacTTtgtAagacagcTTTtg   1296
Consensus
```

Fig. 4: Sequences of second exon of *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.). Compared sequences of *salmons* are underlined.

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Also the results aligned with tree homology DNAMAN program genetics (Fig. 5). Regarding to third exon, we aligned also Salmons sequences and *Rainbow trout*, the results are shown the homology between sequences except *Rainbow trout* were very high.

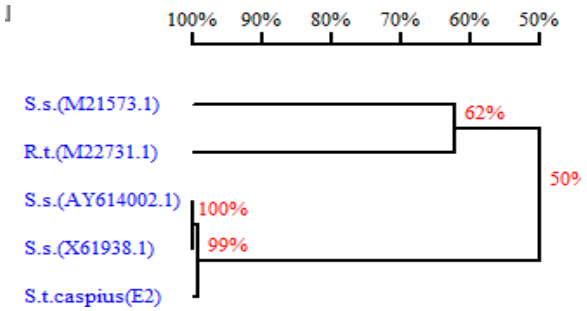

Fig. 5: Homology tree analysis of sequences of the second exon from *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.).

Also, about markers we find repeated A-T-C nucleotides belong to mini and microsatellite in the length of third exon that same second exon. The length of third exon in the *Salmo trutta caspius* is 117 nucleotides while in the *Salmons* was 160 and *Rainbow trout* was not there any nucleotides in the length. The third exon mutations between *Salmons* was very poor (Fig. 6).

```
S.s. (M21573.1)      TGATTTTGTGCAGGAAGGCACCCTGTTGTCTGATGAACGC   1444
R.t.(M22732.1)       ....................................       278
S.s. (AY614002.1)    TGATTTTGTGCAGGAAGGtACCCTGTTGcCTGATGAACGC   1420
S.s. (X61938.1)      TGATTTTGTGCAGGAAGGtACCCTGTTGcCTGATGAACGC   1472
S. t.caspius(E3)     ............GAAGGCACCCTGTTGcCTGATGAACGC     27
Consensus
S.s. (M21573.1)      AGACAGCTGAACAAGATATTCCTGCTGGACTTCTGTAACT   1484
R.t.(M22732.1)       ....................................       278
S.s. (AY614002.1)    AGACAGCTGAACAAGATATTCCTGCTGGACTTCTGTAACT   1460
S.s. (X61938.1)      AGACAGCTGAACAAGATATTCCTGCTGGACTTCTGTAACT   1512
S. t.caspius(E3)     AGACAGCTGAACAAGATATTCCTGCTGGACTTCTGTAACT     67
Consensus
S.s. (M21573.1)      CGGACTCCATCGTGAGCCCAATCGACAAGCAGGAGACTCA   1524
R.t.(M22732.1)       ....................................       278
S.s. (AY614002.1)    CtGACTCCATCGTGAGCCCAATCGACAAGCttGAGACTCA   1500
S.s. (X61938.1)      CtGACTCCATCGTGAGCCCAATCGACAAGCttGAGACTCA   1552
S. t.caspius(E3)     CtGACTCCATCGTGAGCCCAATCGACAAGCttGAGACTCA    107
Consensus
S.s. (M21573.1)      GAAGAGTTCAGTAAGTTACC   1544
R.t.(M22732.1)       ..................     278
S.s. (AY614002.1)    GAAGAGTTCAGTAAGTaACC   1520
S.s. (X61938.1)      GAAGAGTTCAGTAAGTaACC   1572
S. t.caspius(E3)     GAAGAGTTCA..........   117
Consensus
```

Fig. 6: Sequences of the third exon in *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.) compared sequences are underlined.

We also discussed third exon with homology tree (Fig. 7), that results denoted among of homology between *Salmons* except *Rainbow trout* and *Salmo salar* accession number (M21573.1). About fourth, fifth and sixth exon also there were same results, but regarding *Rainbow trout* we found strong homology in the fourth exon also the length of fourth exon in the *Salmo trutta caspius* was 157 nucleotides

and in the *Rainbow trout* was 165 nucleotides that only seven nucleotides was different that it is not significantly.

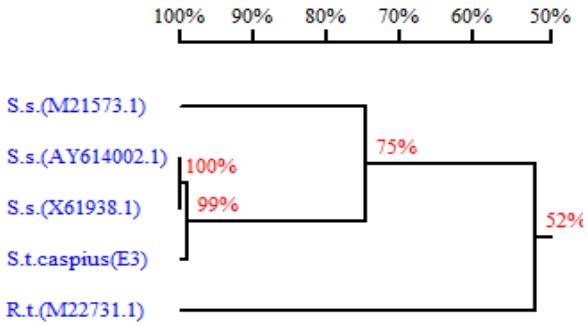

Fig. 7: Homology tree analysis of sequences of the third exon in *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.).

However the results are shown the exon fragments of *Salmons* were longer than *Salmo trutta caspius* but the homology in the all of fragments were high. (Figs. 8, 9, 10). These fragments also aligned with tree homology DNAMAN program (Figs. 11, 12, 13). The fragments had regions of mini and microsatellites that were repeated A-T-C nucleotides.

```
S.s. (M21573.1)       ...ACACACACAGGTCCTGAAGCTGCTCCATATCTCTTTC   2015
R.t.(M22732.1)        ...gaAgAgttcaGTCCTGAAGCTGCTCCATATCTCTTTC    359
S. t.caspius(E4)      ............GTCCTGAAGCTGCTCCATATCTCTTTC     27
S.s.( AY614007.1)     cacACACACACAGGTCCTGAAGCTGCTCCATATCTCTTTC   2360
S.s. (AY614002.1)     cacACACACACAGGTCCTGAAGCTGCTCCATATCTCTTTC   2359
Consensus
S.s. (M21573.1)       CGCCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGG   2055
R.t.(M22732.1)        CGCCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGa    399
S. t.caspius(E4)      CGtCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGa     67
S.s.( AY614007.1)     CGtCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGa   2400
S.s. (AY614002.1)     CGtCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGa   2399
Consensus
S.s. (M21573.1)       CCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGAT   2095
R.t.(M22732.1)        CCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGAT    439
S. t.caspius(E4)      CCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGAT    107
S.s.( AY614007.1)     CCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGAT   2440
S.s. (AY614002.1)     CCATCTCCAACAGCCTAATGGTCAGAAACTCCAACCAGAT   2439
Consensus
S.s. (M21573.1)       CTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAATCTG   2135
R.t.(M22732.1)        CTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAAcCTG    479
S. t.caspius(E4)      CTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAAcCTG    147
S.s.( AY614007.1)     CTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAAcCTG   2480
S.s. (AY614002.1)     CTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAAcCTG   2479
Consensus
S.s. (M21573.1)       CTCATCAAGGTAAAGAAAGG   2155
R.t.(M22732.1)        CTCATCA.............    486
S. t.caspius(E4)      CTCATCAAGG..........    157
S.s.( AY614007.1)     CTCATCAAGGTAAAGAAAGG   2500
S.s. (AY614002.1)     CTCATCAAGGTAAAGAAAGG   2499
Consensus
```

Fig. 8: Sequences of the fourth exon in *Salmo trutta caspius* , *Salmo salar* (S.s.)and *Rainbow trout* (R.t) compared sequences are underlined.

```
S.s. (M21573.1)       CTCTCTCTGTCTCCCACAGGGGAGCCAGGATGGCGTACTG   3341
R.t.(M22732.1)        ..................AGGGGAGCCAGGATGGCGTACTG    509
S.s. (AY614002.1)     CTCTCTCTGTCTCCCACAGGGGAGCCAGGATGGCGTACTG   3635
S. t.caspius(E5)      ...................GGGAGCCAGGATGGCGTACTG     21
S.s. (X61938.1)       CTCTCTCTGTCTCCCACAGGGGAGCCAGGATGGCGTACTG   3675
Consensus

S.s. (M21573.1)       AGCCTGGATGACAATGACTCTCAGCATCTGCCTCCCTACG   3381
R.t.(M22732.1)        AGCCTGGATGACAATGACTCTCAGCATCTGCCcCCCTACG    549
S.s. (AY614002.1)     AGCCTGGATGACAATGACTCTCAGCAgCTGCCcCCCTACG   3675
S. t.caspius(E5)      AGCCTGGATGACAATGACTCTCAGCAgCTGCCcCCCTACG     61
S.s. (X61938.1)       AGCCTGGATGACAATGACTCTCAGCAgCTGCCcCCCTACG   3715
Consensus

S.s. (M21573.1)       GGAACTACTACCAGAACCTGGGGGGCGATGGCAACATCAG   3421
R.t.(M22732.1)        GGAACTACTACCAGAACCTGGGGGGCGAcGGCAACgTCAG    589
S. t.caspius(E5)      GGAACTACTACCAGAACCTGGGGGGCGAcGGCAACgTCAG    101
S.s. (X61938.1)       GGAACTACTACCAGAACCTGGGGGGCGAcGGCAACgTCAG   3755
Consensus

S.s. (M21573.1)       GAGAAACTACGAACTGTTGGCCTGCTTCAAGAAGGACATG   3461
R.t.(M22732.1)        GAGgAACTACGAgCTGTTGGCCTGCTTCAAGAAGGACATG    629
S.s. (AY614002.1)     GAGgAACTAtGAgtTGTTGGCCTGCTTCAAGAAGGACATG   3755
S. t.caspius(E5)      GAGgAACTACGAgtTGTTGGCCTGCTTCAAGAAGGACATG    141
S.s. (X61938.1)       GAGgAACTAtGAgtTGTTGGCCTGCTTCAAGAAGGACATG   3795
Consensus
```

Fig. 9: Sequences of the fifth exon in *Salmo trutta caspius*, *Salmo salar* (S.s.) and *Rainbow trout* (R.t.) compared sequences are underlined.

```
S.s. (M21573.1)       TCCCCAAGGTTGAGACCTACCTGACCGTCGCTAAGTGCAG   3719
R.t.(M22732.1)        .....AAGGTTGAGACCTACCTGACCGTCGCcAAGTGCAG    667
S. t.caspius(E6)      ........GTcGAGACCTACCTGACCGTCGCcAAGTGCAG     32
S.s.( AY614007.1)     cCCCCAAGGTcGAGACCTACCTGACCGTCGCcAAGTGCAG   4003
S.s. (AY614002.1)     cCCCCAAGGTcGAGACCTACCTGACCGTCGCcAAGTGCAG   4011
Consensus

S.s. (M21573.1)       GAAGTCGCTGGAGGCCAACTGCACTCTGTAAACATGGGCT   3759
R.t.(M22732.1)        GAAGTatCTGGAGGCCAACT..................     687
S. t.caspius(E6)      GAAGTCaCTGGAGGCCAACTGCACTCTGTAg.........     63
S.s.( AY614007.1)     GAAGTCaCTGGAGGCCAACTGCACTCTGTAgACgTGGGCT   4043
S.s. (AY614002.1)     GAAGTCaCTGGAGGCCAACTGCACTCTGTAgACgTGGGCT   4051
Consensus
```

Fig. 10: Sequences of the sixth exon in *Salmo trutta caspius*, *Salmo salar* (S.s.) and *Rainbow trout* (R.t.) compared sequences are underlined.

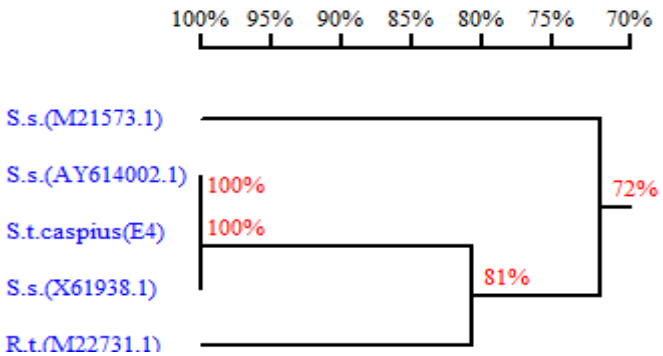

Fig.11: Homology tree analysis of sequences of the fourth exon in *Salmo trutta caspius*, *Salmo salar* (S.s.) and *Rainbow trout* (R.t.).

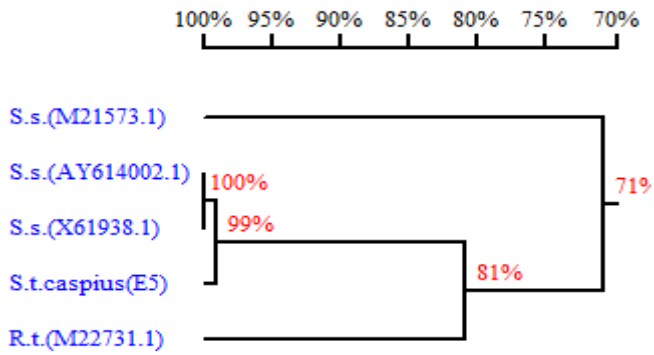

Fig. 12: Homology tree analysis of sequences of the fourth exon in *Salmo trutta caspius*, *Salmo salar* (S.s.) and *Rainbow trout* (R.t.).

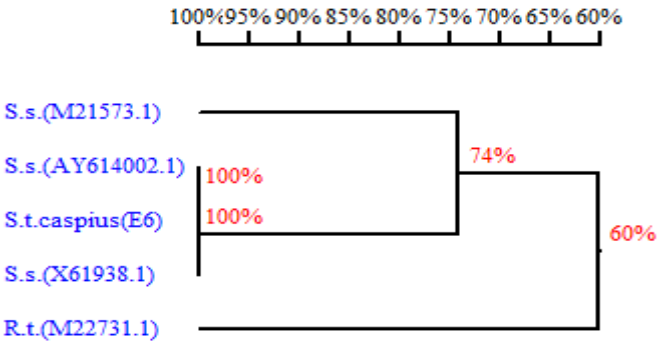

Fig.13: Homology tree analysis of sequences of the fourth exon in *Salmo trutta caspius* , *Salmo salar* (S.s.) and *Rainbow trout* (R.t.).

The ATG codon started in the same region from *S.t.caspius*, S.s. (AY614002.1 and X61938.1), but for S.s. (M21573.1) and R.t. there was no ATG start codon in this region.

The homology between *Salmo trutta caspius* and *Salmo salar* Accession numbers, AY614002.1and *Salmo salar*, Accession number, X61938.1 was almost 100%, and between *Rainbow trout* and *Salmo salar*, accession number M21573.1 was almost 62%. That the rate of varioation was almost high.

The homology sequences, except R.t. were be specific high. The repetitive sequences of nucleotides (underlined fragments) in *S. salar* and *S.t.caspius* denote there are mini and microsatellites in the length.

The homology between *Salmo trutta caspius* and *Salmo salar* Accession numbers, AY614002.1and *Salmo salar*, Accession number, X61938.1 was almost 100%, and between *Rainbow trout* and *Salmo salar*, Accession number, M21573.1 62%. That the rate of varioation was almost high.

The homology between *salmo salar* and *salmo trutta caspius* was high but regards *Rainbow trout* (R.t.) the homology was low. The repetitive sequences of nucleotides (underlined fragments) in *S. salar* and *S.t.caspius* denote there are mini and microsatellites in the length.

Homology between *Salmo trutta caspius* and *Salmo salar* Accession numbers, AY614002.1and *Salmo salar*, X61938.1 was almost 100%, and between *Rainbow trout* and *Salmo salar*, accession number M21573.1 was almost 75%. That the rate of varioation was almost high.

The homology between *salmo salar* and *salmo trutta caspius* and *Rainbow trout* (R.t) was high. The repetitive sequences of nucleotides (underlined fragments) in *S. salar*, *S. t. caspius* and *Rainbow trout* denote there are mini and microsatellites in the length.

The homology between *salmo salar* and *salmo trutta caspius* and *Rainbow trout* (R.t.) was high. The repetitive sequences of nucleotides (underlined fragments) in *S. salar*, *S .t. caspius* and *Rainbow trout* denote there are mini and microsatellites in the length.

The homology between *salmo salar* and *salmo trutta caspius* and *Rainbow trout* (R.t.) was high. The repetitive sequences of nucleotides (underlined fragments) in *S. salar*, *S.t.caspius* and *Rainbow trout* denote there are mini and microsatellites in the length.

The homology between *Salmo trutta caspius* and *Salmo salar* Accession numbers, AY614002.1 and *Salmo salar*, X61938.1 was almost 100% , and between *Rainbow trout* and *Salmo salar*, accession number M21573.1 was almost 81%. That the rate of varioation was almost high.

The homology between *Salmo trutta caspius* and *Salmo salar* Accession numbers, AY614002.1 and *Salmo salar*, X61938.1 was almost 100%, and between *Rainbow trout* and *Salmo salar*, accession number M21573.1 was almost 81%. That the rate of varioation was almost high.

The homology between *Salmo trutta caspius* and *Salmo salar* Accession numbers, AY614002.1 and *Salmo salar*, X61938.1 was almost 100%, and between *Rainbow trout* and *Salmo salar*, accession number M21573.1 was almost 81%. That the rate of varioation was almost high.

DISCUSSION

This study describes the detection of variation GH gene at single copy nuclear genes in *Salmo trutta caspius.* Therefore the Growth hormone gene is a potential target for studies of genetic variation in connection with studies of growth traits (Gross *et al.,* 1995; Gross *et al.,* 1996;Harvey *et al*., 1995). We studied variation within exons of GH1 gene by amplification and sequencing of the full length of GH gene, especially regions of exons. For getting full sequences of exon we designed six pair of primers from first to end of GH gene. In related to we selected from all of *Salmonids* GH gene, specially, *Salmo salar* and *Rainbow trout*, around eleven full sequences GH that reported Gene Bank. These specific primers followed to synthesis around 1.9 kbp for *Salmo trutta caspius*. The results were interestingly for us. Because the each six exons in *Salmo trutta caspius* had been high homology with ten sequences of *Salmo salar* exception of *Salmo salar* Accession number M21573.1. That we could not understand about it, however among of homology between Salmons sequences were very high. Regarding *Rainbow trout*, GH gene has 1171 bp mRNA sequence in the Gene Bank. However the length of sequence in the *Rainbow trout* was shorter than exons of *Salmo trutta caspius* but the homology approximately was significant. The discussion regarding exons fragment with a specific primers pair followed by eleven sequences *Salmo salar* and also one sequence from *Rainbow trout* in the Gene Bank reported. Regarding the sequences of GH gene, the among of variation of exon fragments are very important, The relationships between among the genera in the subfamily *Salmoninae* have been controversial. The analyses of this project and other same projects showed that phylogenesis based on the GH exons strongly supported a sister relationship between the genus *Salmoninae* and other bony fishes. However we should be more researches regarding the markers of genetic related to *Salmons*.

CONCLUSION

We have identified regions of growth hormone gene in *Salmo trutta caspius* that could be a potential genetic markers for *Salmonids*. Moreover, the species of *Salmo trutta caspius* in Caspian Sea is very rarely. So, the

studies regards these fishes are very important. Differences in trait association of the genetic markers may exist among different populations. More tests are needed in other populations of bony fishes to variety associated effects of genetic marker, as well as the effects of the other polymorphisms in the growth hormone and growth hormone gene receptor genes. However the growth hormone gene traits more will be transformed from paternal, So, is better the studies on the other genes markers for example mitochondrial genomics that is associated with maternal traits (Breatchez *et al.,* 1992; Ovenden *et al*., 1993).

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