JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2014. Vol. **8**(6), p. 4281-4288

Detection of Single Nucleotide Polymorphism in Growth Hormone Gene of *Salmo trutta caspius*

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(Received: 11 August 2014; accepted: 21 October 2014)

In this research, I studied on the single nucleotide polymorphism (SNPs) of Growth hormone gene (GH) in *Salmo trutta caspius*. GH gene in *Salmo trutta caspius* have been six exons and five introns the full length. Single nucleotide polymorphism (SNPs) of Growth hormone gene (GH) were compared between *Salmo trutta caspius* and *Salmo salar* by BLAST- NCBI-Network system. Following to BLAST program, were found three gaps in full length of GH gene, first gap originated to 960 bp (*Salmo trutta caspius*) and 1610 bp (*Salmo salar*), second gap originated to 2033 bp (*Salmo trutta caspius*) and 4206 bp (*Salmo salar*), third gap 1318 bp (*Salmo trutta caspius*) and 2637 bp (*Salmo salar*). The all of gaps were not math with together, but the similarity of nucleotides between gaps were high. In this study we found SNPs in full length of GH gene when compared between *Salmo trutta caspius* and *Salmo salar* by BLAST- NCBI-Network system. According my research were found 22 single nucleotide mutations in first gap and 12 single nucleotide mutations in second gap, however there were not any single nucleotide mutation in third gap. For generally similarity of GH gene between *Salmo trutta caspius* and *Salmo salar* is high (98%).

Key words: *Salmo trutta caspius,* single nucleotide polymorphism, Growth hormone gene, *Salmo salar*.

The *Salmonid* species such as *Salmo trutta trutta, Salmo trutta fario, Salmo salar* and *Salmo trutta caspius* and many *Salmonid* species were used for industrial economics, social and environmental importance **(**Gross and Nilsson, 1995). *Salmo trutta caspius* is an important and crucial species of *Salmonids* those rarely in date. These teleost were lived in the Caspian Sea and they migrated for the rivers of connected to the Caspian Sea. According the report of Saadati, 1977, *Salmo trutta caspius* have two forms in Iran: the native *Brown trout* (freshwater populations) and *Caspian Salmon* (sea-run populations) in Namak Lake basins and the Caspian Sea, respectively. The Sea-run Caspian *Salmon* attains a larger size than the freshwater populations. It attains 51 kg and 1.24 m but most of the ones seen in Iran were 10-15 kg (Walczak, 1972 cited in Coad, 2008; Fazeli *et al.* 2011). According above reports the mature populations of *Salmo trutta caspius* can be matured up to 51 kg that function of growth hormone is important for them. Growth hormone gene in *Salmonids* have been two types, type 1 and 2 (McKay. 2004) which diverged at least 30 million years (MY) ago (Devlin, 1993).The two growth hormone (GH) genes are only duplicated pair of genes in *Salmonids* for which both loci have been sequenced (Agellon *et al.* 1988). Growth hormone (GH) plays a very important role in many

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regulatory, metabolic and developmental processes in various vertebrate tissues. The other function of GH gene also were subjected on the phylogenetic analysis such as allozyme polymorphism, specifically studied populations with high and low LDH-5 allele between the two groups using this marker. (Bernatchez and Wilson 1998; Aurelle 1999), mitochondrial genomics (Avise *et al*. 1987; Rezaei and Akhshabi, 2011; Rezaei *et al.* 2011; Rezaei and Akhshabi, 2012; Rezaei *et al.* 2012; Rezaei 2012a; Rezaei 2012b; Hynes *et al.* 1996).

GH gene in *Salmo trutta caspius* were sequenced and deposited in Genbank, accession number, JN241634.1 (Rezaei *et al.* 2011). GH gene in *Salmo salar* has been 6581 bp, accession number (X61938.1) and *Salmo trutta fario*, 2513 bp, accession number (JX155657.1), the results analysed with BLAST- NCBI-Network system, were showed, high similarity between species of *Salmonids* using GH gene, hence we will concluded they are common descent from *Salmonids*.

In this study we aimed discuss on the composite of GH gene in *Salmo trutta caspius* with other *Salmonids*, like *Salmo salar* and *Salmo trutta*, by subjected to single nucleotide polymorphism (SNPs). There are some studies on the SNPs markers in *Salmonids* such as mitochondrial and nuclear DNA, neutral genes, and selected genes such as MHC (Kim *et al.* 1999; Werner *et al.* 2004). They found SNPs between *Salmonids* species like Chinook *salmon*, *Chum salmon*, and *Sockeye salmon*. According the objective of this study, there are two questions? 1) Is there any SNPs in GH gene? 2) If yes, how many SNPs were found in the length of GH gene in *Salmo trutta caspius*?

MATERIALS AND METHODS

Samples

The samples of *Salmo trutta caspius* has taken on the fish, August 2013 from the Rivers of Tonekabon- Iran, these samples including bloods and muscles has taken from three old age females and then anaesthetized with 50 mg of MS222/1. The process of sequencing were found following to;

DNA preparation

DNA genomic from *Salmons* were extracted by kit of extraction DNA (Chrome DNA extract- CHROMOUS BIOTECH PVT. LTD). The quality of DNA extracted were measured by spectrophotometry instrument. DNA quality was also checked by running the sample in 1.5 percent agarose gel electrophoresis. The DNA samples devoid of smear were used for further study.

Designing pair primers for amplify of GH gene in *Salmo trutta caspius*

In this study we used primers according the sequences of *Salmo trutta truta* and *Salmo salar* that reported in Genbank, NCBI Network system. The above sequences cited probably have been high homology with *Salmo trutta caspius*.

DNA amplification

A 2048 bp full length of the growth hormone gene spanning over six exon and five introns was amplified. After using sequence specific primers, we amplified PCR products of GH gene from gDNA (2.2 kb). In the next step, had done purification of PCR products (by gel elution/ PCR clean up). Finally, sequencing of the PCR products by primer-walking and detection of SNPs on the Chromatogram data when deposited the full length in Genbank.

RESULTS

Designing primers, sequence references and sequencing of GH gene

In this study, we designed three set of primers, from GH gene those reported in Genbank, the sequences followed by Accession nos., M22732.1 (*Oncorhynchus mykiss gairdneri*), X61938 (*Salmo salar* gene for growth hormone I), because probably, those sequences have been high homology with GH gene in *Salmo trutta caspius*, hence it was good chance for designing primers (Table 1; Figure 1).

Sequence data obtained from *Salmo trutta caspius*

The sequence of GH gene in *Salmo trutta caspius* were amplified by PCR termocycler then designed new primers and sequenced the full length of GH gene, these results were showed in Figure 2.

Analysis of GH gene following to single nucleotide polymorphism (SNPs)

According the sequences of GH gene those reported in Genbank, Accession no. M22732.1 (*Oncorhynchus mykiss gairdneri*), X61938 (*Salmo salar*), we analysed in the BLAST-

NCBI- Network system. The results showed that there were SNPs between GH gene in *Salmo trutta caspius* and *Salmo salar* (Figure 3). The results followed by BLAST-NCBI- Network system, were found three gaps in full length of GH gene in *Salmo trutta caspius*. The SNPs analysis were found in first gap, 22 single nucleotide mutations and 12 single nucleotide mutations in second gap when compared both *Salmo trutta caspius* and *Salmo salar.*

Table 1. The three sets primers were used for amplification of full lengh of GH gene (green color). Sequence primer were designed for sequencing of nucleotides (blue color)

Primer	Sequence (5´ to 3´)	Product Size
Fwd Primer(SsGH1):	ACATACTCAACCGACCACCGCACTTTCAAG	910
Rev Primer(SsGH2):	GTGACAGGTCCACTCTGCTATTCA	
Fwd Primer(SsGH3):	GTAAATAGGGAATCTCAAGCTGT	312
Rev Primer(SSGH4):	CTCAAATACTTCTAGTAAGTTGA	
Fwd Primer(SsGH5):	CATCACTAATATTGACTATATCAG	819
Rev Primer(SsGH6):	CAGATTAGGCCTTGCCCTGCACTGA	
Sequence Primer:	ATCTGGTAGAGCCTGACTCCA	

Exons highlighted in the reference sequence

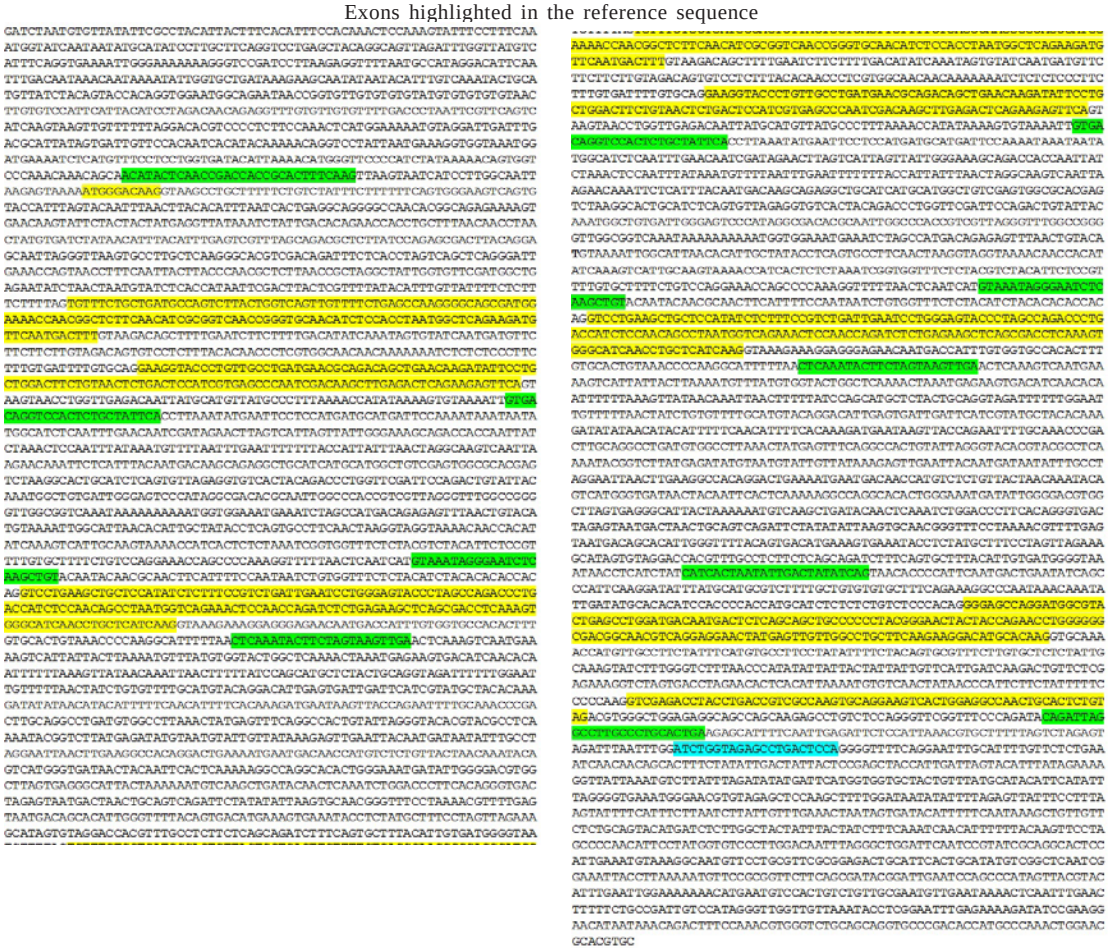

Fig. 1. The sequence of GH gene from *Salmo salar* that reported from Genebank for designing primers subjected to GH gene in *Salmo trutta caspius*. Yellow: Exons, Green: Primers designed for amplification, Blue: Primer designed for sequencing and amplification

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CAAAAAATCATCCCTTGCGCAATTAAGAGTAAAAATGGGACAAGGTAAGCCTGCTTTTTCTGTCTATTTCTTTTT
TTAGTGGGAAGTCAGTGTACCATTTAGTACAGTTTAACTTACACATTTAATCACTGAGGCAGGGGCCAACACGGC
AGAGAAAAGTGAACAAGTATTCTACTACTATGAGGTTATAATCTATTGACACAGAACCACCTGCTTTAACAACCT
AACTATGTGATCTATAACATTTACATTTGAGTCATTTAGCAGACACTCTTATCCAAAGCGACTTACAGGAGCCAT
TAGGGTTAAGTGCCTTGCTCAAGGGCACATCGACAGATTTCTCACCTAGTCAGCTCAGGGATTGAAACCGGTAAC
CTTTCAATTACTTACCCAACGCTCTTAACCGCTGGGCTATTGGTGTACAATGGCTGAGAATATCTAACTAATGTA
TCTCACCATAATTCGACTTACTCGTTTTATACATTTCTTATTTTATTTAATCTCTCTTTTAGTGTTTCTGCTGAT
GCCAGTCTTACTGGTCAGTTGTTTTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTTCAACATCGCGGT
GAACCGGGTGCAACATCTCCACCTAATGGCTCAGAAGATGTTCAATGACTTTGTAAGACAGCTTTTGAATCTTCT
TTTGACATATCAAATAATGTATTAATGATTGTTCTTCTTCTTGTAGACAGTATCCTCTTTACACAACCCCTCGCG
GCTAAAAAAAAACAACAGAAAATCTCTCTCCCTTTCTTTGTGATTTTGTGCAGGAAGGCACCCTGTTGCCTGATG
AACGCAGACAGCTGAACAAGATATTCCTGCTGGACTTCTGTAACTCTGACTCCATCGTGAGCCCAATCGACAAGC
TTGAGACTCAGAAGAGTTCAGTAAGTAACCTGGCTGAGACAATACGCATGGACGCCCTTAAAACCCCTTAAATTT
GGCGGGTTGG----------
TGTAAATAGGGAATCTCAAGCTGTACAATACAACGCAACTTCATTTTCCAATAATCTGTGGTTTCTCTACACACA
CAGGTCCTGAAGCTGCTCCATATCTCTTTCCGTCTGATTGAATCCTGGGAGTACCCTAGCCAGACCCTGACCATC
TCCAACAGCCTAATGGTCAGAAACTCCAACCAGATCTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAACCTG
CTCATCAAGGTAAAGAAAGGAGGGAGAACAATGACCATTTGTGGTGCCACACTTTGTGCACTGTAAACCCCAAGG
CATTTTTAACTCAAATACTTCTAGTAAGTTGAAGTTG----------
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TGCTATATCAGTAACACCCCATTCAATGACTGAATATCGGCCCATTCAAGGATATTTATGCATGTTTCTTTTGCC
GTGTGTGCTTTCAGAAAGGCCCAATAAACAAATATTGATATGCACACATCCATGCATCTCTCTCTGTCTCCCACA
GGGGAGCCAGGATGGCGTACTGAGCCTGGATGACAATGACTCTCAGCAGCTGCCCCCCCTACGGGAACTACTACCA
GAACCTGGGGGGCGACGGCAACGTCAGGAGGAACTACGAGTTGTTGGCCTGCTTCAAGAAGGACATGCACAAGGT
GCAAAACCATGTTGCCTTCAATTTCATGTACCTTCCTATATTTTTTACAGTGCGTTGTTTTTTTGTGCTCTCTAT
TGCAAAGTATCTTTGGGTCTTTAACCCATATATTATTACTATTATTGTTCATTGATCAAGACTGTTCTCGAGAAA
GGTCTAGTGACCTAGAACACTCACATTAAAATGTGTCAACTATAACCCATTCTTCTATTTTTTCCCCCCAAGGTC
GAGACCTACCTGACCGTCGCCAAGTGCAGGAAGTCACTGGAGGCCAACTGCACTCTGTAGACGTGGGCTGGAGAG
GCAGCCAGCAAGAGCCTGTCTCCAGGGTTCGGTTTCCCAGATACAGATTAGGCCTTGCCCTGCACTGAACAGCAT
TTTCGATTGAGATTCTCCATTAAAACATGCTTCTTTTGTTGTGGAGTAAAG
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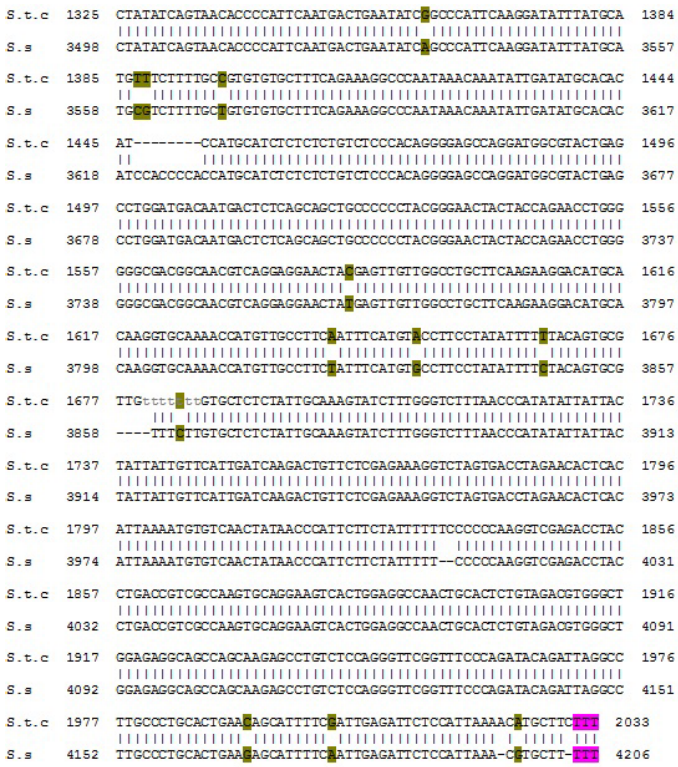
Fig. 2. There are 6 exons in reference sequence. All 6 exons are highlighted in the above sequence data obtained from *Salmo trutta caspius.* Yellow highlighted are exons



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S.t.c  986   TGTAAATAGGGAATCTCAAGCTGTACAATACAACGCAACTTCATTTTCCAATAATCTGTG  1045
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S.s   2296   TGTAAATAGGGAATCTCAAGCTGTACAATACAACGCAACTTCATTTTCCAATAATCTGTG  2355

S.t.c 1046   GTTTCTCTACA--------CA-CACAGGTCCTGAAGCTGCTCCATATCTCTTTCCGTCTG  1096
             |||||||||||        || ||||||||||||||||||||||||||||||||||||||
S.s   2356   GTTTCTCTACATCTACACACACCACAGGTCCTGAAGCTGCTCCATATCTCTTTCCGTCTG  2415

S.t.c 1097   ATTGAATCCTGGGAGTACCCTAGCCAGACCCTGACCATCTCCAACAGCCTAATGGTCAGA  1156
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S.s   2416   ATTGAATCCTGGGAGTACCCTAGCCAGACCCTGACCATCTCCAACAGCCTAATGGTCAGA  2475

S.t.c 1157   AACTCCAACCAGATCTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAACCTGCTCATC  1216
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S.s   2476   AACTCCAACCAGATCTCTGAGAAGCTCAGCGACCTCAAAGTGGGCATCAACCTGCTCATC  2535

S.t.c 1217   AAGGTAAAGAAAGGAGGGAGAACAATGACCATTTGTGGTGCCACACTTTGTGCACTGTAA  1276
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S.s   2536   AAGGTAAAGAAAGGAGGGAGAACAATGACCATTTGTGGTGCCACACTTTGTGCACTGTAA  2595

S.t.c 1277   ACCCCAAGGCATTTTTAACTCAAATACTTCTAGTAAGTTGAA  1318
             |||||||||||||||||||||||||||||||||||||||||
S.s   2596   ACCCCAAGGCATTTTTAACTCAAATACTTCTAGTAAGTTGAA  2637
```

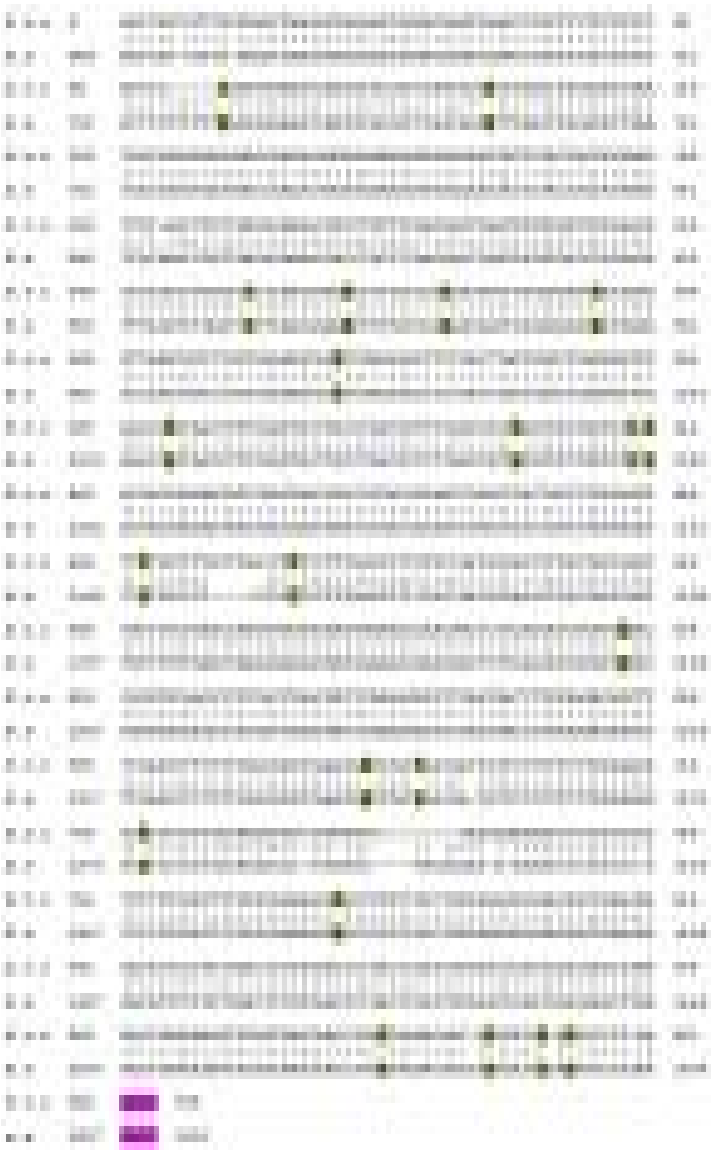

Fig. 3. The alignment of *Salmo trutta caspius* (S.c.t) and *Salmo salar* (S.s) following by BLAST Network system. Dark green color and pink color were SNPs and end of the gaps respectively

DISCUSSION

Why studies on the *Salmo trutta caspius* is important?

Salmo trutta caspius is endemic to the Caspian Sea basin (Bernatchez *et al.* 1992). They migrated to the Rivers of connected to south of Caspian Sea, such as Sardabroud and Cheshme kileh for reproductive and Parr smolt transformation. The mature of *Salmons* has been 51 kg that related to environmental parameters too (Berg, 1948). The *Salmo trutta* registered in Red List of IUCN (2008) that *Salmo trutta caspius* as critically endangered. Their evaluations for the taxon were based on the available population abundance data in the region. According to the report of government in Iran, the Caspian Sea has been pollution and it is very crutial for *Salmons*, pollution in Caspian Sea cause of reduce *Salmons* and infected them. Hence, studies, maintenance and carry out of *Salmo trutta caspius* is important for researchers.

The function of GH gene in *Salmo trutta caspius*

GH gene is synthesized in the somatotrophic axis and is contributed with growth body and reproduction performance (Duan, 1998; Gomez *et al.* 1998) and osmoregulation (Mccormik, 2001). In addition, growth hormone may be the most promising growth-promoting agent in aquaculture (Zohar, 1989), since it is essential for somatic growth and reproduction in bony fishes and osmoregulation in euryhaline fishes (Sciara *et al.* 2006). Among vertebrates, GH is essential for normal growth and is involved in the regulation of several anabolic processes (Xu *et al.* 2001). Furthermore, GH gene is associated with new markers like allozymes and mitochondrial genomics (Marins *et al.* 2003; Chen *et al.* 2004; Pinheiro *et al.* 2008; Rezaei and Akhshabi, 2011; Rezaei *et al.* 2011; Rezaei and Akhshabi, 2012; Rezaei *et al.* 2012; Rezaei, 2012a; Rezaei, 2012b) was used as a molecular tools. In this study we used the single nucleotide polymorphisms (SNPs) for analysis of full length of GH gene in *Salmo trutta caspius*. In Figure 2 for designing of the primers of GH gene we referred to GH gene in *Salmo salar* that reported in Genbank. According the Berg, *et al.* 1962, proposed that *Salmo trutta* had originated from Atlantic Ocean that had been migrated to White Sea and then left to Russia in Caspian Sea. Also they proposed that these *Salmons* is related to deep, and the rivers of around Caspian Sea is very good for passing period of smolt and egg laid by adult *Salmons* those will select the rivers of connected to Caspian Sea, in fact these results introduced and denoted that S*salmo trutta caspius* can be originated from *Atlantic salmons*. In this study we analysed the full length of GH gene in *Salmo trutta caspius*, subjected by SNPs. In Figure 2 and 3 were showed exons and introns of sequences (*Salmo salar* and *Salmo trutta caspius*) that were same both sequences. According the Figure 2, there were six exons for both sequences but the regions of exons between sequences was different, for *Salmo trutta caspius* has been three gaps, first gap originated to 960 bp (*Salmo trutta caspius*) and 1610 bp (*Salmo salar*), second gap originated to 2033 bp (*Salmo trutta caspius*) and 6581 bp (*Salmo salar*), third gap 1318 bp (*Salmo trutta caspius*) and 2637 bp (*Salmo salar*). The all of gaps were not math with together, but the similarity of nucleotides between gaps were high. There are three hypothesis for gaps, 1) According Figure 1 and 2, similarity of exons in both species (*Salmo trutta caspius* and *Salmo salar*) almost were same but about introns was different, however the similarity of introns between species were same but the length of introns in *Salmo trutta caspius* was shorter than *Salmo salar*. May be *Salmo trutta caspius* was originated from *Salmo salar*. Berg *et al*. (1962) proposed that *Salmo trutta* originated from Atlantic Ocean that had been migrated to White Sea and then left to Russia in Caspian Sea, in fact these results introduced and denoted that *Salmo trutta caspius* can be originated from *Salmo salar*. 2) May be *Salmo trutta caspius* has not been originated to *Salmo salar* but exactly similarity of GH gene both species was high. 3) However, GH gene is a marker genetic but for getting exactly result about similarity of species *Salmons*, we should be do on the other marker genetics such as mitochondrial genomics, allozymes and related markers.

Single nucleotide polymorphisms (SNPs) in *Salmo trutta caspius*

In this study we found SNPs in full length of GH gene when compared to *Salmo salar*. According my reseach were found 22 single nucleotide mutations in first gap and 12 single nucleotide mutations in second gap, however there

were not any single nucleotide mutation in third gap. Following these studies, the single nucleotide polymorphism have been reported in economic traits in several fishes, such as *Brown trout* (Gross and Nilsson, 1999), *Bleak* (Gross and Nilsson, 1996), *Atlantic salmon* (Gross and Nilsson, 1999) *Common bream* (Gross and Nilsson, 1996) and *Large yellow croaker* (Ni *et al.* 2012), *Chinook salmon* (Park *et al.* 1995). Therefore, the purpose of the current study was to identify and characterize SNPs in GH gene and then analyze the association between these polymorphisms and growth traits in the mixed pedigrees of *Salmo salar* population. These SNPs would provide basic data for *Salmo trutta caspius*. However, the GH gene denoted for studies on phylogenetic in *Salmons* but it is better research focus on the other markers such as mitochondrial genomics, RAPD, RFLP, mini and microsatellites and Allozymes.

CONCLUSION

In this study we used growth hormone gene in *Salmo trutta caspius* for comparing with other *Salmonids* such as *Onchorhyncus mykiss* and *Salmo salar*.The results showed, there were SNPs between them. According our results, were revealed SNPs (single nucleotide polymorphisms) between *Salmo salar* and *Salmo trutta caspius*, furthermore, we aimed determine full length of mitochondrial genomics of *Salmo trutta fario* and *Salmo trutta caspius* for finding the rate of similarity between them and also other s*almonids* as soon as possible.

ACKNOWLEDGMENTS

This work had financially supported by the Research Council of Islamic Azad University Tonekabon Branch, Iran.

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