Genetic variability of rainbow trout (*Oncorhynchus mykiss*) cultured in Iran using molecular RAPD markers

Afzali, M.[1]; Rahimi Mianji, G[2]*; Gholizadeh, M.[2]

Received: January 2012 Accepted: July 2013

Abstract

In the present study we evaluated the amount and distribution of genetic variation by using RAPD marker variation of 12 markers loci in three broodstock groups of rainbow trout. A total of 47 polymorphic bands were observed in Iranian strain, average number of bands was 10 and average number of polymorphic bands per primer was 3.92. The total detected bands in rainbow trout strain originated from French, was 120 bands with an average number of 10 bands per RAPD primer. A total of 117 amplified were detected in Norwegian population, with an average number of bands and average number of polymorphic bands per primer was 9.75 and 2.58, respectively. Data for observed and effective number of alleles, Nei's genetic diversity and Shannon's information index for all the three populations were 1.31, 1.20, 0.120 and 0.170, respectively. The mean coefficient of gene differentiation value and the estimate of gene flow across the populations were found as 0.299 and 0.171, respectively. The Nei measures of genetic distance and identity between pairs of rainbow trout strains indicate that the strain originated from France and Iran has the highest genetic identity, while the fish originated from Norway and France showed the greatest genetic distance.

Keywords: Genetic variation, Rainbow trout, Polymorphism, RAPD

[DOR: 20.1001.1.15622916.2013.12.3.2.9]

1-Islamic Azad University, Sari Branch, Sari, Iran.

2-Laboratory for Molecular Genetics and Animal Biotechnology, Dept. of Animal Science, Faculty of Animal Science and Fisheries, Sari Agricultural Science and Natural Resources University, P.O. Box, 578 Sari, Iran.

*Corresponding author's email: rahimimianji@yahoo.com

Introduction

Rainbow trout reared in north of Iran are primarily descendants of early imports from Europe. However, industry production and broodstock development within rainbow trout farms in Iran was based primarily on reproduction of these strains. Most existing rainbow trout farms have little information on early origins or pedigrees of their broodstocks. The study of genetic variability is of prime importance for genetic approaches to fish conservation or breeding, which depend on knowledge of the amount of variation existing in within and between broodstock groups. In this work we analyzed the applicability of the Random Amplified Polymorphic DNA (RAPD) as molecular genetic marker to characterize the rainbow trout strains farmed in northern part of Iran, Mazandaran province. RAPD technique has received a great deal of attention from population geneticists because of its simplicity and rapidity in revealing DNA-level genetic variation (Skibinski, 1994). Major drawbacks of RAPD markers in population genetic studies of outbreeding organisms are the potential for reduced reproducibility and that they are dominant. Thus gene frequency estimates for such loci are necessarily less accurate than those obtained with codominant markers such as allozymes and RFLPs (Bardakci, 2001). It has been suggested that two to ten times more individuals need to be sampled for dominant markers to achieve the same degree of statistical power as codominant markers such as allozymes and RFLPs (Lynch and Milligan, 1994). Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. Despite the reproducibility problem, the RAPD method will probably be important as long as other DNA-based techniques remain unavailable in terms of cost, time and labors (Bardakci, 2001). The ability of the RAPD technique to reveal intra-specific variation can be used in screening for the degree of inbreeding in commercial plant and animal species to prevent an increase in the frequency of deleterious recessive alleles in populations (Bardakci, 2001). In fishes, RAPD marker has been successfully used in phylogenetic studies (Prioli et al., 2002; Barman et al., 2003), population structure analysis (Sekine et al., 2002; Almeida et al., 2003), in fishery management and conservation genetics (Bártfai et al., 2003; Hatanaka and Galetti, 2003; Leuzzi et al., 2004). Jamshidi and Kalbassi (2010) used 16 Random amplified polymorphic DNA (RAPD) markers to estimate genetic relationships between two seasonal immigrant forms (namely fall-run and spring-run) of Caspian trout *Salmo trutta caspius*. Their results showed that the total number of RAPD bands produced in spring and fall-run were 162 bands, of which 69 and 53 bands were polymorphic for each population. Also, Nei's genetic identity and genetic distance between spring-run and fall-run populations were 0.9858 and 0.01430, respectively. Genetic variations is a key factor for populations to be able to face future environmental changes and to ensure long term response to selection, either natural or artificial, for traits of economic or cultural interest (Frankham et al., 2004). In Iran, the most of the rainbow trout strains that cultured

[DOR: 20.1001.1.15622916.2013.12.3.2.9]

around the country originate from the Norway, France and Iran. Despite the commercial importance of the rainbow trout in fish breeding industry of Iran, our knowledge on the genetic background of these fish population is generally not very extensive. Since characterization of the populations of a species is essential for effectual fishery management, the study of polymorphism and diversity of these populations is important to select the best broodstocks (Sajedi et al., 2003). The objective of this study was to evaluate the level of genetic diversity within and between of three rainbow trout strains, which are being widely used in fish breeding industry in north of Iran.

Materials and methods

Sample collection

To estimate RAPD variations within and between strains, a total number of 150 individuals (50 per strain) were sampled from different breeding farms of rainbow trout in Mazandaran province with French, Norwegian and Iranian origin. Fin clip was cut from each individual and transferred in dry ice to the molecular genetics laboratory and stored at -20°C until used for assay.

DNA isolation

Total genomic DNA was isolated according to the protocol of Jackson et al. (1991), with some minor modifications. About 50 mg of Fin clip was ground in 500 µL homogenized buffer (20 mM Tris-HCl pH: 7.5, 200 mM NaCl, 20 mM EDTA). Then 100 µL of 10% SDS and 3 µL Proteinase K (20 mg mL^{-1}) were added and the mixture was incubated at 55 °C for overnight. DNA was purified with standard phenol: chloroform extractions, precipitated with ice cold absolute ethanol and resuspended in 50 µL TE (Tris-EDTA pH: 8) buffer. The concentration and quality of purified DNA was assessed by both spectrophotometry and agarose gel electrophoresis, respectively and then samples were stored at -20°C until used in RAPD assay.

RAPD assay

Ethanol-precipitated DNA sample extracted from each individual was used as a template in RAPD procedures. The 23 different decamer oligonucleotides RAPD markers were used for genotyping of rainbow trout fishes in this study (Table 1). Genomic DNA was amplified by PCR and each 25-µl reaction tube consisted of DNA (about 50-100 ng), 10 pmol of each primers, 200 µM dNTP , 1× buffer (10 mM Tris, 50 mM KCl, 0.1% gelatin, pH. 8.4), 2.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase. Amplification was carried out for 35 cycles of 1 min at 95°C, 1 min at 41-45°C, 1 min at 72°C and final extension at 72°C for 10 min. The PCR products were analysed by electrophoresis through 1.5% agarose gel and visualized by ethidium bromide staining. The alleles were scored manually from the photographed gel and the genotypes of each individual fish at the different polymorphic loci were recorded by direct counting.

Analysis of data

RAPD banding patterns were scored visually from ethidium bromide staining agarose gel. For the analysis and comparison of the patterns, a set of

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distinct, well-separated bands were selected. The genotypes were analyzed in the form of binary variables by recording the presence (1) or absence (0) of these bands only, neglecting other (weak and unresolved groups of) bands. Each locus can be treated as a two-allele system, with only one of the allele per locus being amplifiable by the PCR. We also assumed that marker alleles from different loci do not co migrate to the same position on a gel, and that populations are under the Hardy-Weinberg equilibrium (Lynch and Milligan, 1994). Genetic similarity (GS) between individuals i and j was estimated according to the formula given by Nei and Li (1979):

$$GS_{ij} = \frac{2N_{ij}}{(N_i + N_j)}$$

Where N_{ij} is the number of bands common in individuals i and j, and N_i and N_j are the total number of bands in individuals i and j, respectively, with regard to all assay units. Thus, GS reflects the proportion of bands shared between two individuals and ranges from 0 (no common bands) to 1 (all bands identical). Genetic dissimilarity (GD) was calculated as:

$$GD = 1 - GS$$

Nei's unbiased genetic distance was calculated among different rainbow trout strains with all markers, including monomorphic markers. The Nei's unbiased genetic distance is an accurate estimate of the number of gene differences per locus when populations are small. Total genotype diversity among (Ht) and within populations diversity (Hs) were calculated by applying the G-test to allele frequencies at all loci (Nei, 1978). The number of migrants per generation (Nm), which represents inter-population gene flow, was obtained from Gst parameter by means of the formula Nm = (1 - Gst) / (Gst). The similarity matrix was subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) cluster analysis algorithm and a dendrogram was generated. POPGENE software (population genetic analysis) Version 1.31 (Yeh et al., 1999) was used to calculate the genetic parameters.

Results

One hundred fifty genomic DNA samples were selected randomly from among the individuals of 3 rainbow trout strains cultured in North of Iran. These samples were then amplified with a total of 23 random primers (Table 1). Five RAPD markers did not amplify and not show any bands resulted from these markers. Six markers (AOPG13, AOPG14, AOPG16, AOPG17, ACS32 and ACS45) did not produce stable bands and was not included in data set for further analyses. Twelve out of 23 (AOPA2, AOPA3, AOPA5, AOPA10, AOPA12, AOPG3, AOPG5, AOPG7, AOPG8, AOPG9, AOPG10, AOPG13, AOPG14, AOPG15, AOPG16, AOPG17, AOPG18, AOPG20, AOPG21, ACS32, ACS402 ACS45 and AUBC516) which were amplified and generating stable and easy to score and reproducible bands were selected for genotyping of all individuals from three rainbow trout populations (Fig. 1). These 12 markers amplified fragments across all three strains studied (French, Norwegian and Iranian), with the number of bands ranging from 6 (AOPG3) to 13 (ACS40, AUBC516) which varied in size from 200 to 3300 bp. A total of 120 amplified bands in Iranian

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strain, 47 were polymorphic, with an average number of bands and average number of polymorphic bands per primer was 10 and 3.92, respectively. Percentage of polymorphic bands ranged from 20% (AOPA3, AOPG9) to a maximum of 81.81% (AOPG20), with an average of 37.76% polymorphism (Table 2). A total of 117 bands were detected in Norwegian population, with an average number of bands and average number of polymorphic bands per primer was 9.75 and 2.58, respectively. Percentage of polymorphic bands ranged from 9.09% (AUBC516) to a maximum of 50% (AOPG3), with an average of 26.88% polymorphism (Table 3). The total detected bands from rainbow trout strain originated from French, was 120, with an average number of 10 bands per RAPD primer. Percentage of polymorphic bands ranged from 10% (AOPG9) to a maximum of 63.63% (AOPG20), with an average of 25.33% polymorphism (Table 4).

Table 1: Details of primer sequences for 23 RAPD markers employed for genetic characterization in three rainbow trout populations

Primer	Nucleotide sequence (5´- 3´)	GC (%)
AOPA2	TGCCGAGCTG	70
AOPA3	AGTCAGCCAC	60
AOPA5	AGGGGTCTTG	60
AOPA10	GTGATCGCAG	60
AOPA12	TCGGCGATAG	60
AOPG3	GAGCCCTCCA	70
AOPG5	CTGAGACGGA	60
AOPG7	CAACCTGCGG	70
AOPG8	AGAGAGAGC	55
AOPG9	CGAAGCAGCT	60
AOPG10	GGCTGCAGAA	60
AOPG13	CTAGGTCGGA	60
AOPG14	GCCCCTATGC	70
AOPG15	AACTGGACTG	50
AOPG16	CAATGCCGGA	60
AOPG17	GGGATATCGG	60
AOPG18	GGAGTACTGG	60
AOPG20	TGCGGCTGAG	70
AOPG21	ACCTGAACGG	60
ACS32	CCCACGGATC	70
ACS40	GACTGCTCGG	70
ACS45	CACGTCGGAG	70
AUBC516	AGCGCCGACG	80

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Table 2: The total number of bands, polymorphic and monophormic bands, percentage of polymorphic bands of each RAPD markers as obtained from rainbow trout population of Iranian origin farmed in north of Iran

Primers	NTB	NPB	NMB	%PLM
AOPA2	10	3	7	30
AOPA3	11	5	6	45.45
AOPA5	8	2	8	20
AOPG3	6	3	3	50
AOPG5	8	1	7	12.5
AOPG7	11	5	6	45.45
AOPG9	10	2	8	20
AOPG10	9	3	6	33.33
AOPG15	10	3	7	30
AOPG20	11	9	2	81.81
ACS40	13	7	6	53.84
AUBC516	13	4	9	30.76
Mean	10.00	3.92	6.23	37.76

NTB=no. of total bands, NPB=no. of polymorph bands, NMB=no. of monomorph bands, %PLM=% of polymorphism.

Table 3: The total number of bands, polymorphic and monophormic bands, percentage of polymorphic bands of all the DNA samples as obtained from rainbow trout population of Norwegian origin farmed in north of Iran

Primers	NTB	NPB	NMB	% PLM
AOPA2	9	1	8	11.11
AOPA3	11	4	7	36.36
AOPA5	10	3	8	30.00
AOPG3	6	3	3	50.00
AOPG5	8	2	6	25.00
AOPG7	11	5	6	45.45
AOPG9	10	1	9	10.00
AOPG10	8	1	7	12.50
AOPG15	10	3	7	30.00
AOPG20	10	4	6	40.00
ACS40	13	3	10	23.07
AUBC516	11	1	10	9.09
Mean	9.75	2.58	7.25	26.88

NTB=no. of total bands, NPB=no. of polymorph bands, NMB=no. of monomorph bands, % PLM=% of polymorphism.

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Table 4: The total number of bands, polymorphic and monophormic bands, percentage of polymorphic bands of all the DNA samples as obtained from rainbow trout population of French origin farmed in north of Iran

Primers	NTB	NPB	NMB	% PLM
AOPA2	10	2	8	20.00
AOPA3	11	2	9	18.18
AOPA5	10	2	8	20.00
AOPG3	6	1	5	16.66
AOPG5	8	1	7	12.5
AOPG7	10	2	8	18.18
AOPG9	10	1	9	10.00
AOPG10	9	3	6	33.33
AOPG15	10	3	7	30.00
AOPG20	10	7	3	63.63
ACS40	13	3	10	23.07
AUBC516	13	5	8	38.46
Mean	10.00	2.67	7.33	25.33

NTB=no. of total bands, NPB=no. of polymorph bands, NMB=no. of monomorph bands, % PLM=% of polymorphism.

Table 5: Summary of genetic parameters estimate for three strains of rainbow trout farmed in north of Iran using RAPD markers

Populations	Na	Ne	h	I
Iran	1.39	1.25	0.15	0.22
Norway	1.26	1.14	0.09	0.13
France	1.27	1.21	0.11	0.16
Mean	1.31	1.20	0.12	0.17

Na: observed no. of alleles, Ne: effective no. of alleles, h: Nei's genetic diversity, I: Shannon's information index,

Table 6: Summary analysis of genetic variability across all three strains of rainbow trout populations

Parameters	Ht	Hs	Gs	Nm
Across populations	0.163	0.114	0.299	0.171

Ht and Hs: total genotype diversity among and within populations, Gs: mean coefficient of gene differentiation, Nm: estimate of gene flow.

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Table 7: Nei's (1978) genetic distance (below diagonal) and genetic identity (above diagonal), with Lynch and Milligan correction (1994), between three rainbow trout strains farmed in north of Iran

Origin of strain	France	Iran	Norway
France	*****	0.9520	0.9008
Iran	0.0491	*****	0.9055
Norway	0.1044	0.0992	*****

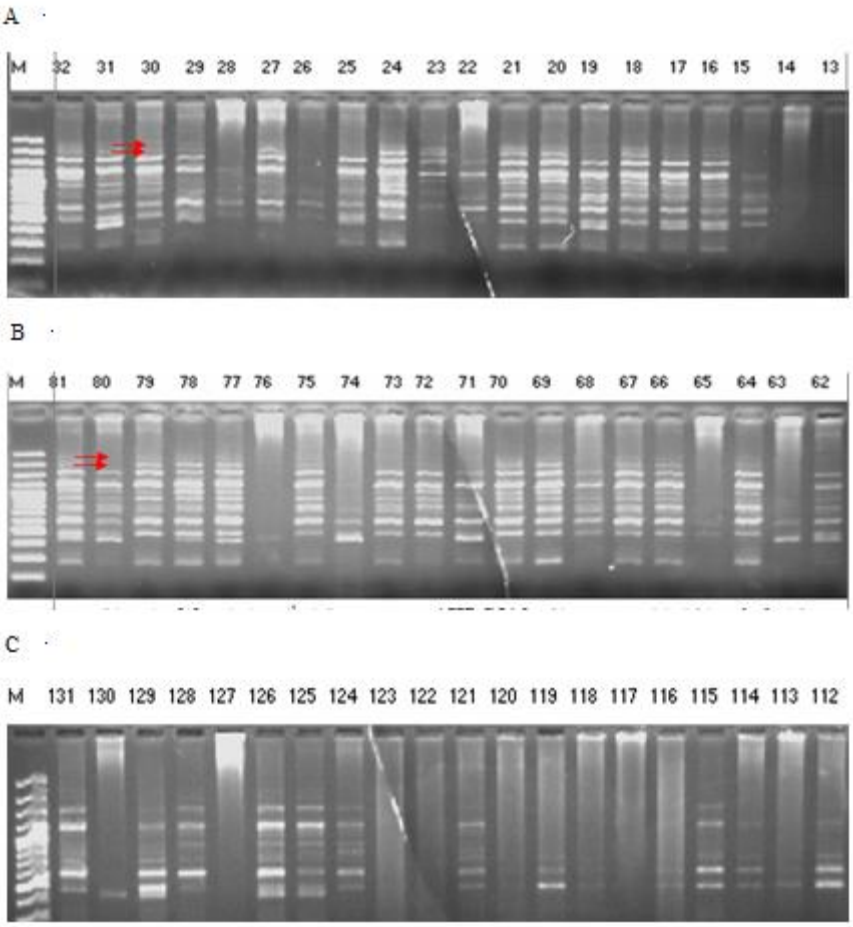

Figure 1: Amplification products of one primer (AUBC516) of: A: French, B: Iranian and C: Norwegian rainbow trout strains. M: Molecular weight markers

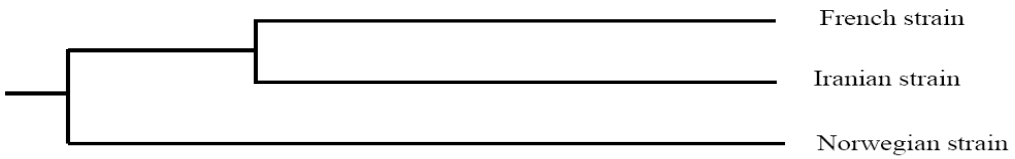

Figure 2: **Dendrogram obtained with UPGMA method based on Nei's DA distance for three rainbow trout strains farmed in north of Iran**

Discussion

It has been shown that domesticated fish strains lose genetic variation through founder effects, genetic drift and selection. Except for fully pedigreed strains, the amount of genetic variation

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lost and the relative importance of these three processes are rarely known (Withler et al., 2005). If a few founder individuals are used to produce offspring and a few of these offspring are used later as founder individuals themselves, this will create an even narrower bottleneck compared to the situation where few founder individuals are used but are replaced continuously with wild-caught individuals (Aho et al., 2006). The rainbow trout farming in Iran have generally been based on small numbers of broodstock. The loss of variation at RAPD loci close to average of 30% across three rainbow trout strains in the present study may attributed primarily to founder effects that occurred with the initiation of rainbow trout farming in north of Iran. The use of such broodstock groups would obviously lead to rapid genetic drift and increased homozygosity if carried on over generations. Data for observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index, for all the three populations were analyzed using twelve RAPD markers and their respective values were found as 1.31, 1.20, 0.120 and 0.170 (Table 5). It has been reported the average of 3.80-4.10 alleles per locus in four populations of Australian rainbow trout, with average heterozygosities of around 0.47-0.58 analyzed by microsatellite markers (Ward et al., 2003). In the present study, the values for total genotype diversity among population (Ht) were 0.163 while within population diversity (Hs) was found to be 0.114. Mean coefficient of gene differentiation (Gst) value and the estimate of gene flow across the populations (Nm) was found as 0.299 and 0.171, respectively (Table 6). The Nei (1978) measures of genetic distance and identity between pairs of rainbow trout strains are given in Table 7 and indicate that the strains originated from France and Iran have the highest identity (0.9520), and the Norway and France populations the greatest genetic distance (0.1044). In the genetic similarity dendrogram constructed on the basis of comparative analysis of the total loci obtained with the 12 RAPD primers across the three populations, two clusters can be seen (Fig. 2). The first was formed by 2 strains from Iran, and France and the second formed by the Norwegian strain. The analyzed data in the present study indicate that the Iranian rainbow trout may be originated from imported French strain in the earlier years. The results of the present study can be seen as a starting point for future research work for detecting the level of within and between strains genetic diversity and to detect genetic relationship among these populations. For this purpose, a larger number of samples of the three strains collected from the whole distribution area should be analyzed and additional codominant DNA markers such as microsatellite tested. Furthermore, in order to design new diagnostic markers more effective in genetic discrimination among strains, specific bands which has been found within each strain could be cloned and sequenced. These studies have given important information in understanding

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genotype relationship, which may further assist in developing and planning breeding programs. Among strains surveyed in this study, the low level of intra and inter-strain genetic diversity were found in rainbow trout populations. The major reasons for loss of genetic variation in broodstock populations may be as a result of bottlenecks and small effective population sizes, due to inappropriate mating designs. It is therefore of great importance that hatchery methods are optimized with regard to mating designs by implementation of pedigreed breeding programmes or identification of individual fish to broodstock and strain of origin for individual companies using molecular markers for preserving genetic variation and passing that variation on to the next generation.

Acknowledgements

We highly appreciate the staff of all fish farms (Kalantari, Mousavi and Miar) for their helpful, cooperation and kind assistance during sample collection.

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