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Evaluating of genetic diversity in three population of rainbow trout (*Oncorhynchus mykiss*) using molecular RAPD markers

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Abstract
In the present study we evaluated the amount and distribution of genetic variation by surveying RAPD marker variation at 12 marker loci in three brood stock groups of rainbow trout. Of the total 120 amplified bands in Iranian strain, 47 were polymorphic, with an average number of bands and average number of polymorphic bands per primer was 10 and 3.92, respectively. The total detected bands in rainbow trout strain originated from French, was 120, with an average number of 10 bands per RAPD primer. 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. Data for observed and effective number of alleles, Nei's genetic diversity, and Shannon's information index for all the three populations and their respective values were found as 1.31, 1.20, 0.120 and 0.170. 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. The obtained low value of genetic variation at the present study indicates that a suitable breeding strategy should be selected in order to increase genetic variation in between and within studied fish populations.
Key words: Genetic variation, rainbow trout, polymorphism, RAPD markers.

Introduction

RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding. This is mainly due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers in a short period compared with other DNA marker methods (Bardakci (2001)). 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)). In fishes, RAPD marker has been successfully used in phylogenetic studies, population structure analysis, in fishery management and conservation genetics (Barman *et a*l. (2003); Almeida *et al*. (2003); Leuzzi *et al*. (2004)). In Iran, the most of the rainbow trout strains that cultured 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. 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.

Material and methods

Sample collection and DNA isolation. To estimate RAPD variations within and between strains, a total number of 50 individuals 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 laboratory and stored at -20°C until used for assay. Total genomic DNA was isolated according to the protocol of Jackson et al. (1991).

RAPD assay. The 23 different decamer oligonucleotides RAPD markers were used for genotyping of rainbow trout fishes in this study. Genomic DNA was amplified by PCR and each 25-µl reaction tube consisted of DNA (about 50-100 ng), primers (10 pmol each), dNTP (200 µM each), 10× buffer (10 mM Tris, 50 mM KCl, 0.1% gelatin, pH. 8.4), $MgCl_2$ (2.5 mM) and *Taq* DNA polymerase (1 U). 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. for each amplification reaction in a pre-programmed thermal cycler. The PCR products were resolved by electrophoresis through 1.5% agarose gel and visualized by ethidium bromide staining. Band patterns were photographed under ultraviolet light.

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 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 comigrate 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 Nei and Li (1979). 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. 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 was used to calculate the genetic parameters.

Results and discussion

One hundreds fifty genomic DNA samples were selected randomly from among the individuals of three rainbow trout strains cultured in North of Iran. These templates were then amplified with a total of 23 random primers. Five RAPD markers did not amplify and no any band resulted from these markers. Six markers did not produce stable bands and was not included in data set for

further analyses. Twelve out of twenty three primers 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. These twelve markers amplified fragments across all three strains studied, with the number of bands ranging from 6 to 13 which varied in size fro 200 to 3300 bp. Of the total 120 amplified bands in Iranian 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% to a maximum of 81.81% with an average of 37.76% polymorphism. 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% to a maximum of 50% with an average of 26.88% polymorphism. 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% to a maximum of 63.63 with an average of 25.33% polymorphism. 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 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. The loss of heterozygosity through inbreeding usually exhibit a weakening of features of adaptation such as survival and fecundity, conservationists assume that a fall in genetic diversity in a population could adversely affect its short-term viability (Leberg (1990)). 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. It has been reported the average of 3.80-4.10 alleles per locus in four populations of Austeralian raibow trouts, 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. The Nei (1978) measures of genetic distance and identity between pairs of rainbow trout strains are given in Table 1 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 (Figure1). 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.

Table 1. 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	**1.00**	0.9520	0.9008
Iran	0.0491	**1.00**	0.9055
Norway	0.1044	0.0992	**1.00**

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 genotype relationship, which may further assist in developing and planning breeding programs.

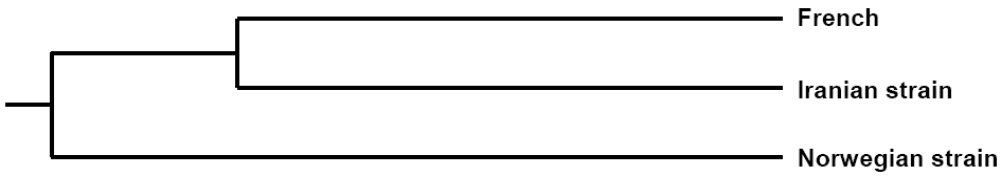

Figure 1. Dendrogram obtained with UPGMA method based on Nei's DA distance for three rainbow trout strains farmed in north of Iran.

Conclusion

In this work we analyzed the applicability of RAPDs as molecular genetic marker to characterize the rainbow trout strains farmed in northern part of Iran, Mazandaran province. However, no such reports on genetic diversity using molecular markers were available in these populations. 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.

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