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of the Institute of Plant Biology and Biotechnology

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MOLECULAR AND GENETIC CHARACTERISTICS OF KAZAKHSTANI RAINBOW TROUT WITH ISSR-PCR ANALAYSIS

Abstract. For the first time a molecular genetic research of the Kazakhstani rainbow trout population *Parasalmo mykiss* presented by samplings from the mountain and foothill rivers of the Almaty region was conducted. From the biopsies tissue of the ventricular finlets kingfish the genomic DNA was isolated for further molecular and genetic research and determination of fish taxonomic belonging to one of the forms of *Parasalmo (Oncorynchus) mykiss* species. The Kamchatka mikizha has some common characteristics with rainbow trout. Therefore, some scientists consider it related to one of rainbow trout type, and others are for the salmon family's independent species. The genomic DNA of Kazakhstani trout, isolated by phenol-chloroform extraction, was analyzed by two ISSR markers. As a result clear polymorphic profiles were obtained, indicating a genetic similarity the Kazakhstani rainbow trout, caught from the Ornek and Ulken-Kakpak rivers, with the Kamchatka rainbow trout. And they also pointed to the high genetic variability of the individuals caught from Tekes river. The obtained preliminary data indicate the possibility of using ISSR markers for studying this species and are the basis for further research in this direction. These results provide a perspective for the possible isolation of the Kazakhstani rainbow trout species into a separate ecological form.

Keywords: rainbow trout, genomic DNA, ISSR-markers, PCR analysis, ecoform.

Introduction. The Kamchatka mikizha *Parasalmo* (*Oncorhynchus*) *mykiss* (Walbaum, 1792) inhabits in the Pacific basin's reservoirs of the Asian and American coasts. Usually the *P.* (*O.*) *mykiss* species is represented by anadrom, estuarine and residential forms. It is known that the anadrom mikizha inhabits the tundra-mountain rivers of the Western and Eastern coasts of Kamchatka, the Okhotsk coast of the continent and the Amur liman. The freshwater mikizha, which is called Rainbow trout, is also widely distributed in Kamchatka [1].

The Kamchatka mikizha was introduced in the 80s of the XX century in the mountain and foothill reservoirs of the southeastern part of Kazakhstan: in Ulken-Kakpak river and Uryukty and Buzumbai lakes in small batches of 2000-3000 pieces per reservoir. In these reservoirs the mikizha grew well and developed for some time [2]. However, the monitoring of Kamchatka mikizha population in Kazakhstani reservoirs was not carried out, despite the fact that it was a valuable commercial species. Six water reservoirs of the Almaty region were investigated in 2015 only, in which the state of the Kazakhstani rainbow trout population (synonym "mikizha") was investigated: the presence, abundance, biometrics and other parameters of fish habitat [3, 4].

However, at present the potential of the species should be assessed not only using standard biometric studies, but also on the basis of accurate information on the genetic structure of the species's population

and the level of their genetic variability. In this connection, there is the necessity arising to investigate the genetic structure of the rainbow trout population in Kazakhstani reservoirs by using modern molecular and genetic markers.

One of the using the molecular markers is the amplification of intermicrosatellite DNA fragments located between two inverted SSR loci of the genome (Inter-Simple Sequence Repeat, ISSR-PCR) [5]. ISSR-typing uses primers complementary to the selected microsatellite motif [6]. Compare with other methods the ISSR-typing is characterized by better reproducibility, and it's used effectively to detect an intraspecific and interspecific genetic variability, identification of species and populations [7].

With reference to the above mentioned the purpose of this work was to reseach the genetic structure of the Kazakhstani rainbow trout population by ISSR-PCR markers.

Objects and methods of research. Control samplings of rainbow trout were obtained from Tekes, Ornek and Ulken-Kakpak Mountain rivers (Almaty region). The catch time was July, as the rainbow trout spawning occurs at a river temperature of 4.8-5.0°C [8]. With the catch each individual was taken from a fragment of the pectoral fin, and then the fish was released into the reservoir.

The fin fragments were fixed in 96% ethanol and transported to the molecular genetics laboratory of the Institute of General Genetics and Cytology for further research.

The genomic DNA from the fin was isolated by standard phenol-chloroform extraction method (1:1), including homogenization in liquid nitrogen [9]. The concentration of isolated DNA was measured with a DNA photometer (Biofotometer Plus, Eppendorf, Germany). For photometric analysis, the adsorption of aqueous DNA solutions was measured at three wavelengths: 260, 280, and 320 nm.

For 80 ng of isolated DNA and 10 μl PCR mixture (PCR Master Mix, Thermo Fisher Scientific, USA) containing specific primers in a concentration of 0.1-0.3 μmol were used for PCR. The primers used in the research were synthesized on the basis of the molecular genetics laboratory of the Institute of General Genetics and Cytology of the Ministry of Education and Science of the Republic of Kazakhstan (Almaty, Kazakhstan), and are presented in Table 1.

Table 1 – Primers used in the amplification of ISSR fragments of rainbow trout

#	5'→3'	T of annealing, ^{o}C	Length b.p.
1	(cag cagcagcagcagcag cag)t	55	22
2	(cag cagcagcag cag)	54	15

The PCR amplification program included the denaturation at 95°C for 5 minutes, then 40 cycles: 95°C for 45 seconds, (54-55)°C for 45 seconds, and 72°C for 45 seconds and the final elongation is 5 minutes at a temperature of 72°C on the Mastercycler nexus gradient (Eppendorf, Germany).

The electrophoretic separation of the reaction products was carried out in a vertical polyacrylamide gel in 1% Tris-acetate buffer, at a voltage of 60 V and a current strength of 90A for 1 hour and 40 minutes. The gel was stained by the dye «SYBR Gold Nucleic Acid Gel Stain» (Thermo Fisher Scientific, USA) with incubation for 20 minutes. Fixation of the result and determination of the size ranges of obtained ISSR fragments was carried out using the Quantum-ST5-1100 gelling system (Vilber Lourmat, France).

Mathematical processing of data was carried out using population genetics methods using the POPGENE Version 1.32 program. To determine the genetic characteristics of rainbow trout populations, the following parameters characterizing the genetic structure were calculated: absolute and effective number of alleles, genetic diversity according to Nei (or expected heterozygosity), information indicator of Shannon variety. The values were calculated on the basis of an analysis of the incidence of DNA ISSR fragments in different populations. Similarities of genotypes were made with the help of computer programs "Statistica 8.0" by the cluster analysis through the dendrograms construction.

Due to the fact that until now the population-genetic research of the Kazakhstani rainbow trout has not been conducted, the choice of ISSR primers was made on the basis of an analysis of foreign literary data. Since the rainbow trout is a freshwater form of the Kamchatka mikizha *Parasalmo* (*Oncorhynchus*) *mykiss*, we selected two primers: (cag)5 and (cag)7t, which were effective for differentiating trout from some geographical groupings (river basins of Kamchatka, Chile and North America) [5]. This made it possible to draw a conclusion about their perspectivity for the Kazakhstani trout.

Results and discussion. Patterns of ISSR fragments for selected primers were obtained for individuals of rainbow trout of all three fish populations from the Tekes, Ornek and Ulken-Kakpak rivers. The ISSR spectra analysis for all three populations revealed 11 amplified DNA fragments, of which 9 (81.82%) were polymorphic. This indicator separately for each population was for the Tekes river – 7, the Ornek river – 4 and the Ulken-Kakpak river – 4. Figure 1 shows the polymorphic DNA fragments for the primer (cag)5.

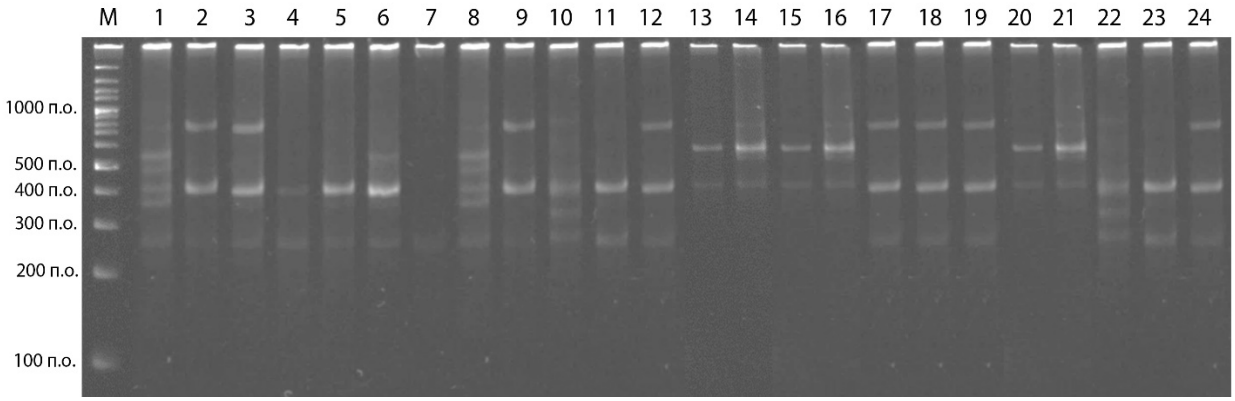

Figure 1 – Polymorphic ISSR profile of the primer (cag) 5: 1-24 – the tested samplings, M – 100 b.p. Plus Ladder marker

The part of polymorphic loci of the rainbow trout population from the Tekes river was 63.64%, Ornek river was 36.36% and Ulken-Kakpak river was 36.36%. This suggests that the population of rainbow trout consisted of genetically more heterogeneous individuals from the Tekes river, while the populations from the Ornek and Ulken-Kakpak rivers were more homogeneous.

The greatest number of polymorphic loci revealed primer (cag)5: the size range of DNA fragments by used primer was from 250 to 1000 b.p.

The analysis of both primers showed that almost all of the tested samplings contained a DNA fragment with a molecular weight of 400 b.p. It can be considered as a species marker for rainbow trout.

Based on the DNA polymorphism's analysis of Kazakhstani rainbow trout allele frequencies were determined by the two ISSR markers. The estimation of the genetic diversity parameters is shown in Table 2 for the three investigated populations.

An estimate of the average of expected heterozygosity separately for the populations showed that the least heterozygous was characterized by for rainbow trout individuals from the Ulken-Kakpak river (0.0936), the largest was from the Tekes river (0.1659). The mean value of the expected heterozygosity for all three populations was 0.1543 (Table 3).

Our data on the expected heterozygosity of rainbow trout from Kazakhstani rivers turned out to be lower than similar statistics obtained by Russian colleagues for mikizha from river basins of the Kamchatka western and eastern coasts. The size of expected heterozygosity in their study was reached to 0.958. There was also a greater heterozygosity for mikizha individuals of Chilean oxbow 0.6851 and North America 0.7145 [10], Alaska from 0.55 to 0.59 [11], California from 0.62 to 0.79 [12].

The lowest level of allelic diversity (ne) was shown for rainbow trout samplings from the Ulken-Kakpak and Ornek rivers: 1.1321 and 1.1493, respectively; the highest one is from the Tekes river sampling. On average this index was 1.2238 alleles per locus. For rainbow trout from seven river systems of Kamchatka it was from 1.9 to 9.8 alleles per locus. In North American samplings of the Kamchatka rainbow trout the average number of alleles per locus was 7.15, in Chilean samplings – 5.2 [10].

Apparently, this circumstance can be explained by the greater rainbow trout populations' isolation in Kazakhstani reservoirs and by the absence of long-range migrations in comparison with the Kamchatka, Chile, and North America populations, as a result of which the influx of "new alleles and genotypes" is declining in the population. Thus, the rainbow trout population (freshwater form of Kamchatka mikizha *Parasalmo* (*O.*) *mykiss*in Kazakhstani reservoirs is genetically less variable than the Kamchatka rainbow trout populations *Parasalmo* (*O.*) *mykiss* from other regions and continents.

Table 2 – Frequency of ISSR markers of research rainbow trout populations

Primer	The size range of fragments, b.p.	Allelic frequency		
		Tekes river	Ornek river	Ulken-Kakpak river
(cag)5	251-300	0.0646	0.2094	0.1548
	301-350	0	0.0646	0.0742
	351-400	0.2094	0	0
	401-450	0.6464	1.0000	1.0000
	451-500	0.1340	0	0
	501-550	0.1340	0	0
	551-600	0.0646	0.2929	0.1548
	601-650	0	0	0
	651-700	0	0	0
	701-750	0.2094	0	0
	751-800	0	0.1340	0.2441
(cag)7t	301-350	0	0.6464	0.0742
	351-400	0.2094	0	0
	401-450	0.6464	1.000	1.000
	451-500	0.1340	0	0
	501-550	0.1340	0	0

Table 3 – Indicators of the genetic diversity of rainbow trout populations.

Population from the river	na	ne	h*	I*
Tekes	1.6364 (0.5045)	1.2465 (0.2741)	0.1659 (0.1620)	0.2675 (0.2422)
Ornek	1.3636 (0.5045)	1.1493 (0.2470)	0.0998 (0.1549)	0.1592 (0.2372)
Ulken-Kakpak	1.3636 (0.5045)	1.1321 (0.2066)	0.0936 (0.1399)	0.1529 (0.2220)
For the total sample size	1.8182 (0.4045)	1.2238 (0.2751)	0.1543 (0.1426)	0.2643 (0.1986)

Note. “na” is the absolute number of alleles per locus; “ne” is the effective number of alleles per locus; h* is genetic diversity according to Nei, or expected heterozygosity; I* is information indicator of the Shannon variety, for all the above parameters, standard deviations are given in parentheses.

The use of ISSR markers (cag)5 and (cag)7t also allowed to evaluate the degree of genetic differentiation of investigated three samplings. Similarities of genotypes were made by the cluster analysis method through the dendrogram construction. Figure 2 shows the genetic relationships of rainbow trout individuals involved in the analysis.

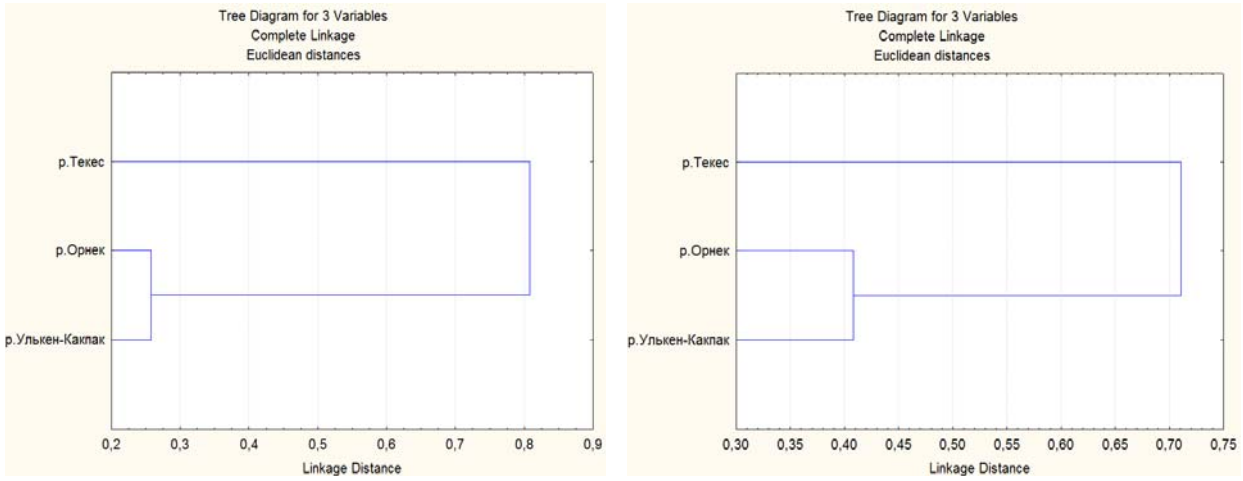

Figure 2 – Dendrogram of genetic distances between research rainbow trout populations by ISSR markers

According to the dendrogram both markers showed the genetic similarities of the rainbow trout individuals caught from the Ornek and Ulken-Kakpak rivers. Individuals from the Tekes river showed heterogeneity in the used ISSR markers. It can be assumed that this population has the highest level of variability in microsatellite markers and differs with a higher level of genetic diversity than other investigated rainbow trout populations in the Ornek and Ulken-Kakpak rivers.

In conclusion we can say that the goals and objectives were performed: when analyzing two inter-microsatellite markers for Kazakhstani rainbow trout we were able to identify the qualitative and quantitative principles of the genetic differences between the samplings from the three investigated Tekes, Ornek and Ulken-Kakpak rivers (Almaty region, Kazakhstan).

This investigation initiated the research of the genetic structure of Kazakhstani populations *Parasalmo* (*O.*) *mykiss* (Walbaum 1792). The preliminary obtained data prove the perspectivity of used ISSR markers for research this species and the importance for planning the further investigations in which an increase in the number of rainbow trout samplings and analyzed genetic markers is expected. It should also be noted that this developed technology could be recommended for practical use in the future.

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ҚАЗАҚСТАНДЫҚ ҚҰБЫЛМАЛЫ БАХТАҚ ПОПУЛЯЦИЯСЫНЫҢ ISSR-PCR АНАЛИЗ НЕГІЗІНДЕ МОЛЕКУЛЯРЛЫҚ-ГЕНЕТИКАЛЫҚ СИПАТТАМАСЫ

Аннотация. Алғашқы рет Алматы облысының таулы және тау бөктері өзендерінің іріктемелерінен келтірілген *Parasalmo mykiss* құбылмалы бахтақ популяциясына молекулярық-генетикалық зерттеу жүргізілді. Құрсақтық жүзбеқанаттарының биопсиялық ұлпаларынан одан кейінгі молекулярлық-генетикалық зерттеу және *Parasalmo* (*Oncorynchus*) *mykiss* микижаның бір формасына жататын балықтың таксономиялық тиесілігін анықтау үшін геномдық ДНҚ алынды. Камчаткалық микижа мен құбылмалы бахтаққа жалпы тән сипаттар бар, сондықтан, бір ғалымдар оны құбылмалық бахтақтың бір түрі десе, басқалары – албырт тұқымдасының өзіндік түрі деп есептейді. Қазақстандық бахтақтың фенол-хлороформдық экстракциясы әдісімен алынған геномдық ДНҚ-сы екі ISSR-маркермен талданған. Нәтижесінде Өрнек және Үлкен-қақпақ өзендерінен ұстап алынған қазақстандық бахтақ дараларының камчаткалық микижасымен генетикалық ұқсастығын көрсететін нақты полиморфтық профильдер алынды. Сонымен қатар, олар Текес өзені дараларының жоғары генетикалық вариабелділігін көрсетті. Алдын-ала алынған мәліметтер ISSR-маркерлердің осы түрді зерттеуге қолдану мүмкіндігін және осы бағытта кейінгі зерттеулерді жүргізуге негіз болатынын көрсетті. Бұл нәтижелер қазақстандық құбылмалы бахтақтың жеке экоформаға жекелену мүмкіндігіне перспективті болып саналады.

Түйін сөздер: құбылмалы бахтақ, геномдық ДНҚ, ISSR-маркерлер, ПЦР-анализ, экоформа.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА КАЗАХСТАНСКОЙ ПОПУЛЯЦИИ РАДУЖНОЙ ФОРЕЛИ НА ОСНОВЕ ISSR-PCR АНАЛИЗА

Аннотация. Впервые проведено молекулярно-генетическое исследование казахстанской популяции радужной форели *Parasalmomykiss*, представленной выборками из горных и предгорных рек Алматинской области. Из биопсированной ткани брюшного плавничка была выделена геномная ДНК для дальнейшего молекулярно-генетического исследования и определения таксономической принадлежности рыб к одной из форм микижи *Parasalmo* (*Oncorynchus*) *mykiss*. Камчатская микижа имеет общие характерные черты с радужной форелью, поэтому одни ученые считают ее одним из видов радужной форели, а другие – самостоятельным видом семейства лососевых. Геномная ДНК казахстанской форели, выделенная методом фенол-хлороформной экстракции, была проанализирована по двум ISSR-маркерам. В результате получены четкие полиморфные профили, указывающие на генетическое сходство с камчатской микижей казахстанских особей форели, пойманных из рек Орнек и Улкен-Какпак. Также они указывали на высокую генетическую вариабельность особей реки Текес. Полученные предварительные данные указывают на возможность применения ISSR-маркеров для изучения данного вида и являются основой для проведения дальнейших исследований в этом направлении. Эти результаты дают перспективу для возможного обособления казахстанского вида радужной форели в отдельную экоформу.

Ключевые слова: радужная форель, геномная ДНК, ISSR-маркеры, ПЦР-анализ, экоформа.

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