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Genetic profiling of the Antoniny–Zozulenets intrabreed type of Ukrainian leather and scaly carp using ISSR markers

Mariutsa A.[1], Borysenko N.[1], Dyman T.[2]

[1] *Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine*

[2] *Bila Tserkva National Agrarian University*

Mariutsa A. E-mail: mariutsa16@ukr.net

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Маріуца А., Борисенко Н., Димань Т. Генетичне профілювання антонінсько-зозуленецьких внутрішньопородних типів української лускатої та рамчастої порід коропа з використанням ISSR-маркерів. Збірник наукових праць «Технологія виробництва і переробки продукції тваринництва», 2026. № 1. С. 135–143.

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Assessment of genetic diversity in aquaculture stocks is essential for maintaining breeding efficiency and preventing genetic erosion in selectively bred fish populations. This study applied inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) markers to evaluate the genetic structure and diversity of the Antoniny–Zozulenets intrabreed type of Ukrainian scaly and leather carp reared at the "Stara Syniava" fish farm. Five trinucleotide ISSR primers ($(CTC)_6C$, $(GAG)_6C$, $(AGC)_6G$, $(ACC)_6G$, $(AGC)_6C$) were used for molecular genetic analysis. A total of 132 DNA fragments were amplified, of which 72.3 % were polymorphic, indicating a considerable level of genetic variability within the studied stocks. The informativeness of the ISSR markers was assessed using polymorphic information content (PIC), percentage of polymorphic bands (PPB), effective multiplex ratio (EMR), marker index (MI), and resolving power (Rp). The mean values of these parameters were PIC=0.123, PPB=72. %, EMR=19.1, MI=2.3, and Rp=9.6, demonstrating the effectiveness of the selected marker system for population genetic analysis. The sizes of amplified DNA fragments ranged from 150 to 1170 bp depending on the primer used. Genetic diversity indices showed moderate variation between the studied groups: Shannon's index reached 0.265±0.017 in leather carp and 0.242±0.018 in scaly carp, while unbiased expected heterozygosity was 0.155±0.011 and 0.144±0.012, respectively. The average number of alleles per locus (Na) was 1.571±0.072 in leather carp and 1.334±0.082 in scaly carp, with effective allele numbers (Ne) of 1.216±0.019 and 1.201±0.019. These results demonstrate that ISSR markers provide a reliable tool for genotyping and monitoring genetic variability in Ukrainian carp populations and can support selective breeding and conservation programs.

Keywords: common carp (*Cyprinus carpio*), ISSR markers, genetic diversity, population genetics, allelic variation.

Problem statement and analysis of recent research. Genetic diversity plays a fundamental role in the stability, adaptability, and long-term productivity of fish populations. In aquaculture, the analysis of population genetic structure is essential for effective broodstock management, conservation of genetic resources, and the development of sustainable breeding programs. Molecular genetic approaches provide powerful tools for identifying hereditary variation and detecting genetic differences among individuals, populations, and breeds. Such analyses enable the detailed characterization of broodstock genetic resources and support the identification and preservation of valuable traits for further selective breeding [12, 26].

In aquaculture systems, long-term artificial selection and intensive cultivation practices can lead to a reduction in genetic variability, loss of alleles, and increased genetic uniformity of cultured stocks. These processes may negatively affect the adaptive potential, disease resistance, and productivity of fish populations. Therefore, continuous monitoring of genetic diversity is considered an essential component of modern aquaculture management [8, 18]. Understanding genetic relationships among populations also helps prevent undesirable hybridization and gene introgression during broodstock formation and selective breeding programs [13, 21].

The application of molecular genetic markers has significantly improved the ability to study population structure and genetic variability in aquatic organisms. DNA-based markers allow the detection of polymorphism at the genome level and provide objective tools for assessing genetic diversity both within and between populations. In aquaculture research, molecular markers are widely used for population genetic analysis, parentage assignment, stock identification, and marker-assisted selection aimed at improving economically important traits [17, 29]. Recent studies emphasize that molecular markers such as microsatellites, single nucleotide polymorphisms (SNPs), and other DNA-based systems play an important role in the genetic improvement and conservation of cyprinid species, including common carp (Cyprinus carpio) [15, 24].

Among the available molecular approaches, inter-simple sequence repeat (ISSR) markers have proven to be an effective tool for detecting DNA polymorphism and assessing genetic diversity in fish populations. ISSR analysis allows the identification of polymorphic loci across the genome and can be used to study genetic relationships between populations, breeds, and lines, as well as to perform individual genotyping. Due to their reproducibility and relatively high level of polymorphism, ISSR markers have been widely applied in population genetics studies, genetic mapping, and the identification of loci associated with economically important traits in aquaculture species [5, 10, 11].

Common carp (Cyprinus carpio) is one of the most important freshwater aquaculture species worldwide and represents a major component of global fish production. The species has long been the subject of genetic and genomic studies aimed at improving productivity, disease resistance, and environmental adaptability. Modern molecular and genomic techniques have significantly expanded opportunities for carp genetic improvement and selective breeding programs [14].

In Ukraine, carp breeding has a long history and represents a key sector of freshwater aquaculture [9]. Ukrainian carp breeds possess valuable biological and productive traits, including high growth rates, adaptability to different environmental conditions, and good reproductive performance. However, maintaining these qualities requires continuous selective breeding and genetic monitoring in order to prevent the loss of valuable hereditary characteristics and preserve the genetic integrity of existing breeds [7].

One of the important structural components of Ukrainian carp breeds is the Antoniny–Zozulenets intrabreed type. This carp type was developed through reproductive crossing of local non-pedigree carp from the Antoniny State Fish Reserve (currently PJSC “Khmelnytskyrybhosp”) with Galician mirror carp. Two phenotypic forms are distinguished within this type: scaly and leather (often referred to in Ukraine as framed carp) [27]. These carp types represent an important genetic basis for the formation of other structural types of Ukrainian carp breeds.

Due to their genetic origin and long history of selective breeding, Antoniny–Zozulenets carp stocks demonstrate high productivity under intensive aquaculture conditions. Selection programs have primarily focused on economically important traits such as growth rate, survival, and reproductive performance. As a result of long-term selective breeding, Ukrainian carp breeds have developed a productive and genetically diverse breeding structure and have been distributed both within Ukraine and abroad [19, 28].

Despite their breeding and economic importance, molecular genetic information on Antoniny–Zozulenets carp types remains limited, particularly regarding the level of genetic diversity and differentiation between scaly and leather forms. Detailed knowledge of the genetic structure of these carp populations is necessary for effective broodstock management, conservation of genetic resources, and further improvement of breeding programs.

Therefore, **the aim of this study** was to assess the genetic diversity and determine the genetic profile of the Antoniny–Zozulenets intrabreed type of Ukrainian leather and scaly carps using ISSR molecular markers.

Material and methods of research. The study was conducted in 2021 using blood samples obtained from the Antoniny–Zozulenets intrabreed type of Ukrainian leather and scaly carp collected at the “Stara Syniava” fish farm located in the Khmelnytskyi region, Ukraine.

Blood samples were collected in vivo from the caudal vein using sterile syringes containing heparin (25 IU mL^{-1}) as an anticoagulant. The samples were centrifuged for 15 min at 3500 rpm, after which plasma and erythrocyte fractions were separated into sterile Eppendorf tubes for further analysis. The samples were transported at 4 °C and stored at –20 °C in the laboratory until analysis. Both fin tissue and blood samples were used as biological material for molecular analysis.

Total genomic DNA was extracted using a commercial Quick-DNA MiniPrep kit with Zymo-Spin IIC columns (200 isolations) according to the manufacturer's protocol (BioLabTech LTD, Ukraine). The concentration and purity of the extracted DNA were determined using a BioPhotometer (Eppendorf, Germany). DNA quality was assessed based on the absorbance ratio at 260 and 280 nm (A260/A280). Only DNA samples with an A260/A280 ratio > 1.75 were used for further analysis.

To investigate the genetic structure of the studied carp groups, ISSR genotyping was performed using five primers targeting DNA fragments containing trinucleotide repeat motifs (Table 1). These ISSR markers have previously been successfully applied in Ukraine for assessing genetic diversity in several fish species.

Polymerase chain reaction (PCR) amplification was performed using a Thermo Scientific thermocycler (Arktik Thermal Cycler, Finland) with Thermo Scientific DreamTaq Green PCR Master Mix (2×). The PCR program included an initial denaturation step at 95 °C for 2 min, followed by 35 cycles consisting of DNA denaturation at 94 °C for 30 s, primer annealing at 55–60 °C (Table 1) for 30 s, and extension at 72 °C for 2 min. The amplification was completed with a final extension step at 72 °C for 10 min.

The amplified products were separated by electrophoresis on a 2% agarose gel. GeneRuler 50 bp DNA Ladder (Thermo Scientific) was used as a molecular weight marker to estimate the size of amplicons. The PCR products were visualized after staining the gel with ethidium bromide (0.5 µg mL^{-1}) under ultraviolet light using a transilluminator.

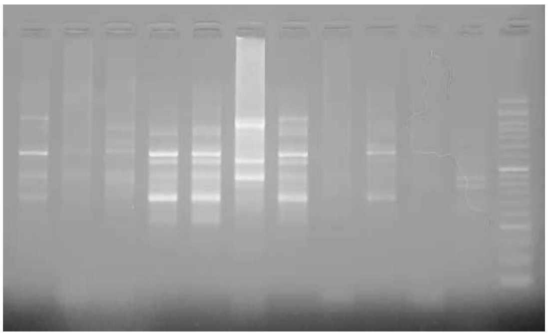
The sizes of amplified DNA fragments were determined using TotalLab software (version 2.01). Genetic diversity parameters were calculated using POPGENE software (version 1.32) [28] and GenAlEx (version 6.5) [20]. The polymorphic information content (PIC) was calculated using the GDdom online tool [1].

The informativeness of ISSR primers was evaluated based on the effective multiplex ratio (EMR), marker index (MI), and resolving power (Rp), calculated according to methodologies developed for dominant molecular markers [22, 23].

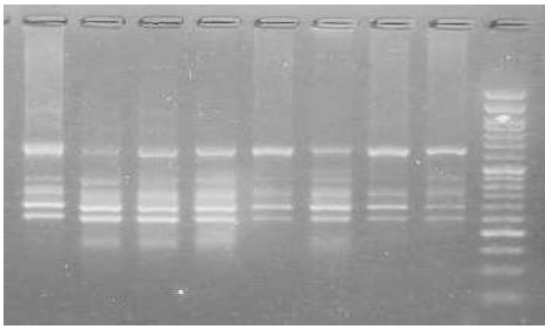
Research results and discussion. The genetic structure of the Antoniny–Zozulenets intrabreed type of Ukrainian leather and scaly carp was analyzed using five ISSR primers. In total, 132 amplicons were detected, of which 72 % were polymorphic, indicating a considerable level of genetic variability within the studied populations (Fig. 1).

Table 1 – **The ISSR primers used in the study for the analysis of the genetic structure of carps**

No	Legend	Primer	Primer sequence 5'→3'	Primer annealing temperature (°C)
1	A	$(CTC)_6C$	5'- CTCCTCCTCCTCCTCCTCC -3'	55
2	B	$(GAG)_6C$	5'- GAGGAGGAGGAGGAGGAGC-3'	58
3	C	$(AGC)_6G$	5'- AGCAGCAGCAGCAGCAGCG-3'	58
4	D	$(ACC)_6G$	5'-ACCACCACCACCACCACCG -3'	58
5	E	$(AGC)_6C$	5'-AGCAGCAGCAGCAGCAGCC -3'	60



a
1 2 3 4 5 6 7 8 9 10 11 M



b
1 2 3 4 5 6 7 8 M

Fig. 1. **Agarose gel of polymorphic DNA amplification profiles of different carp genotypes obtained with ISSR primers:** a – leather carp, 1–8 – different individuals; b – scaly carp, 1–11 – different individuals: M – standard molecular size 50 bp.

The size of amplified DNA fragments ranged from 150 to 1170 bp across the five loci. A relatively narrow range of amplicons was observed for primer A (150–675 bp) in leather carp and primer E (160–650 bp) in scaly carp. In contrast, broader fragment spectra were detected for primer B (263–1000 bp) in scaly carp (SC) and primer D (260–1000 bp) in leather carp (LC).

The highest number of amplicons in the scaly carp group was obtained using primer A ($(CTC)_6C$), which produced 25 amplicons, whereas the lowest number (13) was detected with primer C ($(AGC)_6G$). In the LC group, the highest number of amplicons (23) was detected with primer C ($(AGC)_6G$), while the lowest number (16) was observed with primer A ($(CTC)_6C$).

Using primer A, 25 allelic fragments ranging from 1170 to 244 bp were detected in the SC population. Several fragments (1170, 800, 770, 730, and 600 bp) occurred with a frequency of 16 %, whereas fragments of 1160, 1000, 830, 675, 560, 500, and 250 bp were detected at 12 % frequency. In LC, 16 allelic variants ranging from 675 to 150 bp were identified using the same primer. Fragments of 450 bp and 250 bp showed the highest frequency in the population, while fragments of 645, 600, 350, 335, 244, and 150 bp occurred at a frequency of 12.5 % (Fig. 2).

Primer B produced 14 allelic fragments in the SC group, with sizes ranging from 1000 to 263 bp. Fragments of 300 bp, 285 bp, and 263 bp were detected in 50% of individuals, whereas the 360 bp fragment occurred in 28% of the population. Other fragments were detected at frequencies ranging from 7.1% to 21.4%. In the LC group, 19 allelic variants were detected within a similar size range. Fragments of 400 bp and 350 bp occurred most frequently (31.6 %), whereas fragments of 1000 bp and 450 bp were detected in 21% of individuals.

Primer C generated 13 alleles in the SC group, with fragment sizes ranging from 700 to 280 bp. The most frequent fragments were 700 bp and 440 bp (38 %), followed by 520 bp and 450 bp (31 %) and 365 bp (23 %). In the LC group, 22 alleles ranging from 700 to 240 bp were detected, with fragments of 300 bp (27 %) and 365 bp (23 %) occurring most frequently.

Primer D produced 14 alleles in the SC group, with fragment sizes ranging from 600 to 270 bp. The most frequent fragment was 350 bp (43 %), followed by 450 bp (36 %), 420 bp (29 %), and 460 bp and 370 bp (21 %). In the LC group, 18 alleles were detected within a wider size range (1000–260 bp). Fragments of 400 bp, 320 bp, and 270 bp occurred in 33 % of individuals, whereas fragments of 520 bp occurred in 28 %, and fragments of 420 bp, 339 bp, and 260 bp occurred in 22 %.

Primer E generated 14 alleles in the SC group, with fragment sizes ranging from 600 to 270 bp. In the LC group, 20 alleles were detected with fragment sizes ranging from 470 to 240 bp, with 320 bp fragments occurring most frequently (27 %), while 450 bp and 295 bp fragments occurred in 19 % of individuals.

Several fragments demonstrated potential specificity for particular carp groups. For example, fragments of 300 bp, 285 bp, and 263 bp generated by primer B were detected in 50 % of SC individuals, while fragments of 350 bp (43 %) and 450 bp (36 %) generated by primers D and E were also characteristic of this group. No clear group-specific fragments were identified in the LC group using the selected primers.

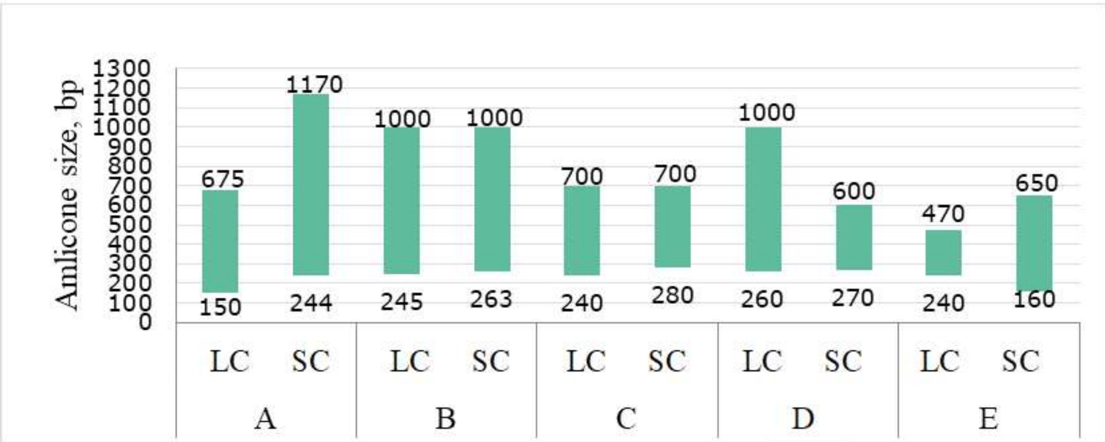


Fig. 2. **The ranges of the obtained amplicons**.

The informativeness of the selected ISSR markers was evaluated using several parameters. The polymorphic information content (PIC) ranged from 0.103 (primer A) to 0.158 (primer C), with a mean value of 0.123 (Fig. 3). The effective multiplex ratio (EMR) varied from 9 (primer C) to 25 (primer A), indicating the highest efficiency of the primer–marker system for primer A. The marker index (MI) showed the highest value for primer A (3.1 in SC; 1.7 in LC) and the lowest value for primer C (1.4 in SC). The resolving power (Rp) was lowest for primer C (3.0 in SC) and primer A (3.4 in LC), whereas higher values were observed for primers A and D (6.526 and 6.329, respectively) (Tables 2 and 3).

The genetic diversity of the studied carp populations was assessed using several parameters. The average number of alleles per locus (Na) was 1.571± 0.072 in LC and 1.334±0.082 in SC. The effective number of alleles (Ne) was 1.216± 0.019 and 1.201±0.019, respectively.

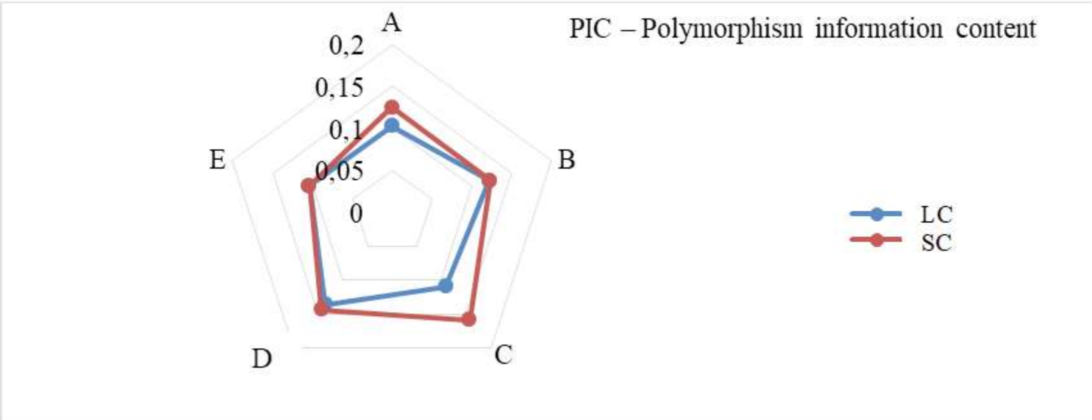


Fig. 3. **Informativeness indicators of the genetic structure of carps.**

Table 2 – **The genetic diversity values of the studied leather carps based on ISSR markers**

Primer	Na	Ne	I	uHe
A	1.231	1.149	0.200	0.120
B	1.760	1.259	0.301	0.188
C	1.917	1.247	0.314	0.191
D	1.467	1.244	0.279	0.178
E	1.481	1.184	0.232	0.141
mean	1.571±0.072	1.216±0.019	0.265±0.017	0.16352±0.012

Table 3 – **Genetic diversity values of the studied scaly carps based on ISSR markers**

Primer	Na	Ne	I	uHe
A	1.923	1.287	0.355	0.222
B	1.360	1.201	0.243	0.152
C	0.750	1.147	0.159	0.104
D	0.933	1.164	0.182	0.117
E	1.704	1.208	0.274	0.165
mean	1.334±0.082	1.201±0.019	0.242±0.018	0.152±0.012

The Shannon diversity index (I) was 0.265±0.017 for LC and 0.242±0.018 for SC. The unbiased expected heterozygosity (He) was 0.155±0.011 and 0.144±0.012, respectively.

Overall, the results demonstrate that the selected ISSR markers are informative for detecting polymorphism and assessing genetic diversity in the Antoniny–Zozulenets intrabreed type of Ukrainian leather and scaly carp.

The analysis of genetic structure using ISSR markers revealed a relatively high level of DNA polymorphism. A total of 132 amplicons were obtained, of which 72 % were polymorphic. The high proportion of polymorphic loci indicates considerable genetic variability within the studied carp populations. Similar results have been reported in previous studies of common carp (Cyprinus carpio), where ISSR markers showed a high level of polymorphism and proved effective for evaluating genetic diversity in aquaculture stocks [5, 11].

Polymorphic information content (PIC) is widely used to evaluate the informativeness of molecular markers in population genetic studies. In the present study, PIC values ranged from 0.103 to 0.158, with a mean value of 0.123. According to Botstein et al. [4], markers with PIC values below 0.25 are considered moderately informative for dominant marker systems such as ISSR. Therefore, the obtained values indicate a moderate level of marker informativeness, which is typical for ISSR markers used in fish population studies. Comparable PIC values have been reported in carp populations, ranging between 0.10 and 0.20 depending on primers and genetic background [3, 11]. These results confirm that the selected ISSR primers are suitable for detecting polymorphism in Ukrainian carp populations.

The effective multiplex ratio (EMR), marker index (MI), and resolving power (Rp) are additional indicators used to evaluate the efficiency of molecular marker systems. In the present study, EMR values ranged from 9 to 25, while the highest marker index values were recorded for primer A. These results indicate that primers A and D were the most efficient for detecting polymorphic loci in the studied carp populations. Similar findings have been reported in ISSR-based studies of cyprinid species, where certain primers showed higher resolving power due to their ability to amplify multiple polymorphic loci simultaneously [3, 23].

Genetic diversity was further evaluated using Na, Ne, Shannon's index (I), and expected heterozygosity (He). The mean number of alleles per locus was slightly higher in LC (Na=1.571) than in SC (Na=1.334), suggesting that the LC population may retain slightly higher genetic variability. Similar patterns have been reported in other carp studies and are often associated with differences in breeding history, selection intensity, and broodstock management [2, 16].

The effective number of alleles (Ne) showed similar values in both groups (1.216 in LC and 1.201 in SC), indicating a relatively balanced distribution of allele frequencies within populations. Comparable values have been reported in carp populations analyzed with dominant markers, reflecting moderate genetic differentiation in cultured stocks [3].

The Shannon diversity index also indicated moderate genetic diversity in both groups. Slightly higher values were observed in LC (I=0.265) than in SC (I=0.242). Shannon's index reflects both allelic richness and evenness and is widely used as an additional measure of genetic variability. The obtained values are consistent with those reported for carp populations in Eastern Europe and Asia, where I typically ranges from 0.20 to 0.40 depending on marker systems and population structure [11, 17].

Expected heterozygosity (He) is one of the key indicators of genetic diversity. In this study, unbiased He values were 0.155 in LC and 0.144 in SC, indicating a moderate level of genetic diversity in both populations. Similar heterozygosity levels have been reported in cultured carp populations, where He generally ranges from 0.10 to 0.20 when dominant markers are applied [2, 16]. The slightly higher heterozygosity in LC may reflect differences in breeding history or a broader genetic base.

The identification of several fragments occurring at relatively high frequencies in SC individuals suggests potential group-specific genetic features within the Antoniny–Zozulenets intrabreed type. Such fragments may serve as useful molecular markers for population identification and monitoring of genetic structure in breeding stocks. However, the absence of clearly specific markers for LC indicates that additional molecular markers may be required for more precise differentiation between these carp forms.

Overall, the results of the present study demonstrate that ISSR markers are effective tools for analyzing genetic polymorphism and assessing genetic diversity in Ukrainian carp populations. The obtained genetic diversity parameters indicate that both scaly and leather carp of the Antoniny–Zozulenets intrabreed type maintain a relatively stable genetic structure with moderate levels of polymorphism. These findings are important for broodstock management and for the development of breeding programs aimed at preserving the genetic diversity of Ukrainian carp breeds.

The use of molecular genetic markers for monitoring the genetic composition of aquaculture stocks is increasingly recognized as an important component of sustainable fish breeding. Therefore, the application of ISSR markers in studies of Ukrainian carp populations provides valuable information for the conservation of genetic resources and the improvement of selective breeding strategies in aquaculture.

Conclusions. The present study assessed the genetic diversity and molecular characteristics of the Antoniny–Zozulenets intrabreed type of Ukrainian leather and scaly carp using ISSR markers. The analysis revealed a relatively high level of DNA polymorphism, with 132 amplified fragments identified, of which 72 % were polymorphic. These results indicate considerable genetic variability within the studied carp populations.

The selected ISSR primers demonstrated moderate to high informativeness for analyzing the genetic structure of the investigated groups. The polymorphic information content (PIC) averaged 0.123, while the effective multiplex ratio (EMR), marker index (MI), and resolving power (Rp) confirmed the efficiency of the marker system, particularly for primers A and D. These primers were the most efficient for detecting polymorphic loci and differentiating individuals within the studied populations.

Genetic diversity parameters also indicated a moderate level of variability. The average number of alleles per locus (Na) and the effective number of alleles (Ne) were slightly higher in leather carp (LC) than in scaly carp (SC), while Shannon's diversity index and expected heterozygosity values were similar in both groups. These findings suggest that the studied populations maintain a relatively stable genetic structure despite long-term selective breeding.

Overall, the results confirm that ISSR markers are suitable tools for assessing genetic diversity and monitoring the genetic structure of Ukrainian carp breeds. The obtained data provide a useful basis for broodstock management, conservation of genetic resources, and further improvement of selective breeding programs in carp aquaculture.

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Генетичне профілювання антонінсько-зозуленецьких внутрішньопородних типів української лускатої та рамчастої порід коропа з використанням ISSR-маркерів

Маріуца А., Борисенко Н., Димань Т.

Оцінювання генетичного різноманіття в аквакультурних популяціях є важливим для підтримання ефективності відтворення та запобігання генетичній ерозії селекційних груп риб. У дослідженні застосовано ISSR-PCR маркери для оцінювання генетичної структури та різноманіття антонінсько-зозуленецьких внутрішньопородних типів української лускатої та рамчастої порід коропа, вирощених у господарстві «Стара Синява».

Для молекулярно-генетичного аналізу використано п'ять тринуклеотидних ISSR-праймерів: (CTC)$_6$C, (GAG)$_6$C, (AGC)$_6$G, (ACC)$_6$G, (AGC)$_6$C). Усього ампліфіковано 132 фрагменти ДНК, з яких 72,3 % були поліморфними, що свідчить про високий рівень генетичної мінливості в межах досліджуваних популяцій.

Інформативність ISSR-маркерів оцінювали за показниками вмісту поліморфної інформації (PIC), відсотка поліморфних смуг (PPB), ефективного мультиплексного коефіцієнта (EMR), індексу маркерів (MI) та роздільної здатності (Rp). Середні значення становили: PIC=0,123, PPB=72,3 %, EMR=19,1, MI=2,3 та Rp=9,6, що підтверджує високу ефективність обраної маркерної системи для популяційно-генетичних досліджень.

Довжина ампліфікованих фрагментів ДНК варіювалася в межах 150–1170 п.н. залежно від використаного праймера. Індекси генетичного різноманіття засвідчили помірну варіабельність між досліджуваними групами: індекс Шеннона становив 0,265±0,017 у рамчастого коропа та 0,242±0,018 у лускатого коропа, тоді як очікувана гетерозиготність – 0,155±0,011 та 0,144±0,012 відповідно. Середня кількість алелів на локус (Na) дорівнювала 1,571±0,072 у рамчатого коропа та 1,334±0,082 у лускатого, а ефективне число алелів (Ne) – 1,216±0,019 та 1,201±0,019 відповідно.

Отримані результати свідчать, що ISSR-маркери є надійним та інформативним інструментом для генотипування і моніторингу генетичної мінливості популяцій коропа в Україні та можуть бути ефективно використані у селекційних програмах і заходах зі збереження генетичного різноманіття.

Ключові слова: короп звичайний (*Cyprinus carpio*), ISSR-маркери, генетичне різноманіття, популяційна генетика, алелі.

ORCID iD:
Mariutsa A. https://orcid.org/0000-0001-5678-2660
Borysenko N. https://orcid.org/ 0000-0001-5031-5682
Dyman T. https://orcid.org/0000-0002-6428-1476