Evaluation of The Genotype of Barbus Luteus (Heckel, 1843) in Dhi Qar and Basra by ISSR-PCR Technique

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Abstract

This study was conducted in the Laboratory of Molecular Genetics / Marine Sciences Center / University of Basrah, from 1/1/2019 to 1/1/2020. Tissue samples of Hamri fish were randomly collected from the governorates of Basra (Al-Qurna District) and Dhi Qar (Al-Nasr District). A total samples was 60, including 30 samples from Basra and 30 from Dhi Qar. The results show that the ISSR12 primer has a genetic morphology 100%, gave the molecular weights in Dhi Qar Governorate (73 and 255) and in Basra Governorate (66, 65). Primer No. (2) also took shape in the province of Dhi Qar (166, 186, 244), and in the province of Basra (194, 237). The primer (ISSR12) gave 8 bundles, with a molecular weight ranging from (73,255) to samples taken from the Dhi Qar and Basra regions. As for the primer (ISSR8).

Keywords: Genotype, Barbus luteus (Heckel, 1843), Dhi Qar, Basra, ISSR-PCR technique.

1. Introduction

Fisheries is one of the most important natural resources that mankind has exploited since ancient times through fishing, global fish production is about 75 million tons each year, developing countries contribute about 48% , it can achieve food security, and at the same time is an important source of income and economy [1]. Humans have used fish as food over the ages, as it is an easy to digest food, rich in proteins and fats and as a medicine for many diseases, used as a prophylaxis for pulmonary tuberculosis and impaired liver function. Fisheries is one of the living aquatic resources and it is one of the renewable natural resources, which has the ability to renew itself year after year through natural reproductive processes [2].

Genetic markers are powerful tools for researching the genetic relationship between breeds [3]. Molecular markers are polymorphic, evenly distributed across the genome, are inexpensive, rapidly detectable, and reproducible, there are many molecular markers that have proven their potential in the diagnosis of different types of animals such as protein conformation, random amplification DNA-ISSR conformation, RFLP, VNTR, SNP, STR and micro-sequencing [4].

The ISSR-PCR technique was developed by Williams et al. [5]. It is based on PCR polymerase chain reaction using similar primers for random regions on the genome, this technique has been successfully used in studying population structure and genetic variance, the main advantages of this technique are its ease, low cost and speed of application compared to other techniques, this technique was used to determine the relationships between different breeds and individuals and to formulate breeding strategies, as well as being used to control pedigree records for animals [6, 7]. The Barbus luteus fish belongs to the family Cyprinidae, the economically important and widespread species in Iraq, spreads in most inland water bodies, especially the central regions [8]. This study seeks to know the evaluation of the genetic variation of Hamri fish and study its genetic relationships using the ISSR-PCR technique.
2. Materials and Methods

2.1 Study site

This study was conducted in the Laboratory of Molecular Genetics / Marine Sciences Center / University of Basrah, from 1/1/2019 to 1/1/2020. Tissue samples of Hamri fish were randomly collected from the governorates of Basra (Al-Qurna District) and Dhi Qar (Al-Nasr District). A total samples was 60, including 30 samples from Basra and 30 from Dhi Qar.

2.2 Devices

Table 1: The equipment used in the study.

| No. | Devices                                | Origin and manufacturer          |
|-----|----------------------------------------|----------------------------------|
| 1   | Refrigerated Centrifuge (15000rpm)     | Germany/ Hettich                 |
| 2   | Centrifuge (Spin)                      | Germany/ Edwards                 |
| 3   | pH meter                               | Europe/ HANNA                    |
| 4   | sensitive scale                        | Germany/ Denver                  |
| 5   | sterilizer                             | Japan/ Hirayama                  |
| 6   | Gel Documentation Device               | USA/ Voternix                    |
| 7   | water bath                             | UK/ Grant OLS200                  |
| 8   | Shaker (Vortex)                        | Germany/ Heidolph                |
| 9   | water distiller                        | USA/ Omega scientific            |
| 10  | Electrophoresis and electrical power supply | UK/ Cleaver Scientific       |
| 11  | microwave oven                         | China/ Monlinex                  |
| 12  | Nano Drop 2000 (Spectrophotometer)     | U.S.A/ Thermo Scientific         |

2.3 Sample collection

Tissue samples were collected as one sample for each animal, adequate care has been taken in collecting tissue samples to avoid any contamination. 25 mg per sample. The samples were placed in a 1.5 mL Eppendorf tube and kept at -20°C until the DNA extraction process.

2.4 Extraction of deoxyribonucleic acid (DNA) from blood.

DNA was extracted from fish tissue samples taken from the dorsal muscles using the Genomic DNA Mini Kit (Tissue) according to the following steps with some necessary modifications:

- 25 mg tissue was taken and placed in a 1.5 ml Eppendorf tube and 200 μl of Phosphate buffered saline – PBS solution was added.
- 20 μL Proteinase K and 20 μL RNase were added.
- Mix the mixture calmly and carefully so as not to damage the DNA and incubate at room temperature for two minutes.
- 200 μl of Genomic lyses/ Binding buffer were added and shaken.
- Samples were incubated for 30 minutes in a water bath at 60°C.
- 200 μl of 100% absolute ethanol were added and the samples were shaken by shaking for several seconds.
- 640 μl was taken from the filtrate and then transferred to the centrifuge at 10,000 cycles for one minute.
- The filtrate was taken and the precipitate was discarded, then 500 μl of wash buffer 1 was added and then centrifuged at 10,000 cycles for one minute.
- The precipitate was removed, then 500 μl of Wash buffer 2 was added, then centrifuged at 10,000 cycles for 3 minutes.
- Eliminate the precipitate and take the filtrate into a new 1.5 ml Eppendorf filter, then add 150-200 μl of Elution buffer, then centrifuge 10,000 cycles for one and a half minutes, after which the samples are kept at -20 °C until use.
2.5 Measurement of the concentration of deoxyribonucleic acid (DNA) with a Nano Drop

The amount of DNA was measured by the Nano Drop device supplied by the American company Thermo Scientific to know the size of the genome (DNA concentration in ng/µl), by following the following steps:

- Clean the bottom and top measuring surfaces of the Nano Drop before use by a clean cloth and very little distilled water.
- A titration was carried out by applying 1 µl of 1X TE Buffer solution by a micro-pipette to the lower surface and lowering the container arm to the upper surface, then the word Blank was pressed for calibration.
- After the titration process is completed, remove 1X TE Buffer solution from the measurement surfaces using a clean dry cloth, then write the name of the sample in the Sample ID box, and choose the DNA type of the sample and the unit of concentration ng/µl.
- Place 1 microliter of the DNA sample to be measured on the lower surface, lower the container arm on the upper surface, then press the word Measure on the wavelengths 260nm to read the DNA and 280nm to read the protein.
- Clean the measuring surfaces using a dry cloth and repeat steps 2, 3 and 4 each time the amount of DNA of a new sample is measured.

2.6 Detection of deoxyribonucleic acid (DNA)

Before carrying out the process of DNA detection, some operations were performed, as follows:

2.6.1 Preparation of agarose gel

After preparing and washing the basin of the relay gel, and attaching the comb to one end, put the plastic pieces on the edges of the sink. Migration is carried out on agarose gel at a concentration of 1%, i.e. 0.25 g of agarose is dissolved in 25 ml of 1X TBE solution, put it in a beaker and heat it in the microwave for 1.5 minutes, until a clear color was obtained for the mixture, an amount of (2 µl) of ethidium bromide dye was added. After the agarose has cooled to 50°C, taking into account the shaker of the beaker well, the dye will be homogeneous with the mixture, then the gel was poured into the transfer trough and left to solidify [9].

2.6.2 Electrophoresis to reveal the genome of study fish

To identify the products of the DNA extraction process, the technique of electrophoresis on agarose gel was used. The migration gel pool was immersed in the main basin containing 1X TBE migration solution. After the gel has hardened and the comb and plastic pieces are lifted, 6 µl of the DNA product is mixed with 2 µl of the dye Bromophenol blue. The mixture was injected into the holes, and after the injection process was completed, the electrodes were connected to the power supply. The electric current was fixed at 80 volts and 65 mA and left the gel until the transfer of DNA and Bromophenol blue dye from the pit (negative electrode) to the other side (positive electrode). After the migration process, the gel was examined in a UV apparatus to see the DNA bands interfering with the ethidium bromide dye.

![Figure 1: Detection of the genetic material of Barbus luteus fish.](image)
2.6.3 ISSR-PCR Technique

Prepared the materials for the PCR technique, placed in a bowl containing pieces of ice for the purpose of protecting it from heat, process was carried out in a sterile and clean place in a special PCR Cabinute, contains UV rays to sterilize micropipettes, tubes and tubes, taking into account, wear special sterile gloves when working. Prepare the PCR reaction mixture in a 100 μl Eppendorf tube, the final volume of components was 25 μl, then the tubes were placed in a small centrifuge, for the purpose of mixing the reaction components for 30 seconds (Table 2), then it was placed in the thermal cycler. Three molecular primers (Table 3) were used in this study to find out the genetic variance [10].

| chemical  | Master Mix | Primer | DNA template | Distilled water | Final volume |
|-----------|------------|--------|--------------|----------------|--------------|
| Volume    | 12.50      | 2.00   | 5.00         | 5.50           | 25.00        |

Table 3. List of ISSR primers used for the genetic diversity study in different fish species.

| S. N. | Primer name | Sequences 5’-3’ | GC (%) | Tm (°C) |
|-------|-------------|-----------------|--------|---------|
| 1     | ISSR12      | CTCTCTCTCTCTCTCTAC | 50     | 54      |
| 2     | ISSR8       | ACACACACACACACACT | 48     | 51      |
| 3     | M1          | AGAGAGAGAGAGAGGT | 50     | 50      |

3. Results and Discussion

Table (4) show that the most prominent results obtained from the amplification of DNA genetic material into Hamri fish are as follows:

The ISSR12 primer has a genetic morphology 100%, gave the molecular weights in Dhi Qar Governorate (73 and 255) and in Basra Governorate (66, 65). Primer No. (2) also took shape in the province of Dhi Qar (166, 186, 244), and in the province of Basra (194, 237). The primer (ISSR12) gave 8 bundles, with a molecular weight ranging from (73,255) to samples taken from the Dhi Qar and Basra regions. As for the primer (ISSR8), it gave 10 with the same molecular weight to the two regions, and this indicates the presence of almost identical genetic conformations.

Table 4. Evaluation of the genetic variance of Barbus luteus in the governorates of Dhi Qar and Basra using ISSR-PCR technique.

|     | P1 | P2 |
|-----|----|----|
| Dhi Qar | Basra | Dhi Qar | Basra |
| 73 | 66 | 186 | 194 |
| 255 | 65 | 244 | 237 |

From the results recommend that there be an expansion of the study of local (Iraqi) genetic assets to all livestock and fisheries, to create an archive for reference in the event of dependence in breeding, multiplication and preservation of what is threatened with extinction of wild and aquatic animals for this country. According to the mentioned results, there are multiple genetic formations genetically but stable phenotypically and in order to detect the difference voluntarily, necessary to perform the sequences technique to strip the DNA of these local fish. It was concluded that the used technique showed a morphological diversity estimated at 100% in the genetic variance between the study samples and it ranges widely in biodiversity and bimolecular research in the field of animal production and fish farming. Whereas, the phenotypic correlation between two traits is representative of the contrast between the phenotypic values of those two traits, and that this correlation is due to the environment, heredity and the common factors between them [11].

References

[1] Andersskog, B. (1966) Iraq-Preliminary fishery survey. Report to the government. Report FAO/UNDP(TA), (TA 2226), p 12.
[2] Craig S. and L.A. Helfrich (2002) Understanding Fish Nutrition, Feeds and Feeding. Cooperative Extension Service publication 420-256. Virginia State University, USA.
[3] Caetano-Anolles G.; B.J. Basam and P.M. Gresshoff (1991) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology 9: 553-557.

[4] Rafalski, A.; S. Tingey and J.G.K. Williams (1992) Random amplified polymorphic DNA (RAPD) markers. Plant Molecular Biology Manual, 423-429.

[5] Afnan Nema Mnaty and Jamal Naser Abedalrahman, 2021, Study of Sediments Accumulated of Tigris River in Al-Kut Barrage and Their Effect on Engineering Criteria of River Sections, Al-Qadisiyah Journal For Agriculture Sciences, 11, 1, 1-9. doi: 10.33794/qjas.2021.168284.

[6] Crowhurst, R.N.; B.I. Hawthorne; E.H. Rikkerink and M.D. Templeton (1991) Differentiation of Fusarium solani f. sp. cucurbitae races 1 and 2 by random amplification of polymorphic DNA. Current Genet. 20 391-396.

[7] Rao, K.B., K.V. Bhat and S.M. Totey (1996) Detection of species-specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD). Genet. Anal., 13: 135-138.

[8] Al-Tamimi, L.M.A. (2004) Environment, life and assessment of fish community in the Euphrates River near the Musayyib power station. PhD thesis, College of Agriculture, University of Basra, 147 pages.

[9] Williams J.G.K.; J.A. Rafalski and S.V. Tingey (1993) Genetic analysis using RAPD markers. In: R. Wu (Eds.) Methods in Enzymology. Orlando, FL: Academic Press. Vol. 218, 704–740.

[10] Mohammadi, A. and S. Bahramikia (2019) Molecular identification and genetic variation of Alternaria species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods. Curr Med Mycol., 5(2): 1-8.

[11] Falconer, D.S. (1989) Introduction to Quantitative Genetics, Ed. 3 Longmans Green/John Wiley & Sons, Harlow, Essex, UK/New.