Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 1811 – 1826 (2025)
www.ejabf.journals.ekb.eg

Strong Expression of Foreign DNA Fragments Following Intramuscular Injection into the African Catfish, *Clarias gariepinus*

Samy Y. El-Zaeem[1*], Ragaa A. Abdallah[2], Hussein A. Adam[2], Amany M. Hendy[1*], Hanaa Z. Tawfik[2]

[1]Animal and Fish Production Department, Faculty of Agriculture- Saba-Basha, Alexandria University, Alexandria, Egypt

[2] Faculty of Fish and Fisheries Technology, Aswan University, Aswan, Egypt

[*] **Corresponding Author: selzaeem@alexu.edu.eg , amany.m.hindy@alexu.edu.eg**

ARTICLE INFO

Article History:
Received: July. 20, 2025
Accepted: Sep. 4, 2025
Online: Oct. 3, 2025

Keywords:
Clarias gariepinus,
DNA direct intramuscular injection,
Genetically modified fish,
African catfish,
glutathione reductase gene,
ISSR

ABSTRACT

Gene transfer methods (transgenesis) enable the transfer of traits between organisms to develop new lineages with improved aquaculture performance. Consequently, genetically modified organisms (GMOs) offer potential for genetic enhancements, including faster growth, increased production and efficiency, disease resistance, and broader ecological adaptability. Therefore, this study aimed to investigate the fate of foreign DNA fragments isolated from common carp (*Cyprinus carpio*) following direct intramuscular injection into African catfish (*Clarias gariepinus*). Three treatment doses [65µg/ 0.1ml/ fish (T1), 130µg/ 0.1ml/ fish (T2), and 195µg/ 0.1ml/ fish (T3)] were administered via intramuscular injection. The sequence of the *glutathione reductase* gene (*gsr*) was used as a molecular marker to validate the DNA transfer approach in the gonads and muscles of African catfish (*C. gariepinus*). The DNA sequencing results observed a variation in the sequence similarity scores to the sequence of the *C. carpio gsr* gene in all injected African catfish. Furthermore, to assess the genetic diversity between the same fish before and after intramuscular injection, Inter-Simple Sequence Repeat (ISSR) analysis using five primers revealed a reduction in genetic diversity, particularly in fish receiving the highest dose (195 µg/0.1 ml/fish), as indicated by lower Jaccard's similarity coefficients (0.56) compared to the other groups. These results, along with the other results from the DNA sequencing, suggested that the same fish, after receiving the exact dose, became genetically modified. In conclusion, this study provides valuable preliminary insights into the direct intramuscular injection of foreign DNA that would be an efficient, time- and cost-effective method for transferring DNA fragments between organisms to create new genetic lines.

INTRODUCTION

Clarias gariepinus (Burchell, 1822), the African catfish, is one of the most important fish farmed in various regions of the world. The North African catfish, *C. gariepinus*, is very important to aquaculture and fisheries. Distributed throughout Africa and found natively in Minor Asia, it has also been used for aquaculture in Europe, America, and Southeast Asia. This kind of fish lives in lakes, rivers, and dams because it is a freshwater fish. Aquaculture has favored the use of *C. gariepinus* owing to its ease of

cultivation, rapid growth rate, disease resilience, capacity to endure a range of temperatures, and low requirements for dissolved oxygen **(Oyeleye *et al.*, 2016; Hendy, 2019; Shourbela *et al.*, 2020)**.

Selective breeding and other conventional genetic improvement techniques have not always been successful in increasing the diversity and viability of farmed fish. The challenge of distinguishing desirable features from undesirable traits has resulted in a reduction in genetic gains through selective breeding. To further enhance this industry's growth, new technology must be adopted **(Levy *et al.*, 2000)**. Genetically modified fish is a common term that refers to whole fish whose DNA has been artificially changed by directly combining (or deleting) single or multiple genes to introduce or modify the target character. Various techniques have been implemented to produce genetically modified fish. More importantly, the introduction of different types of fluorescent proteins has greatly expanded the range of applications of transgenic fish. Presently, transgenic fish are widely used as experimental models for medical science, pharmacology, and ecotoxicology **(Wang *et al.*, 2021)**.

One of the main objectives of inserting new elements into the fish genome is to create superior commercial strains for aquaculture **(Maclean & Laight, 2000; El-Zaeem, 2001; Melamed *et al.*, 2002; Sarmaşık, 2003; Tsai HuaiJen, 2003)**. The production of cultured organisms could be enhanced by genetically modified fish. This technology makes it feasible to introduce new features or enhance existing ones in ways that traditional breeding techniques cannot **(Aleström, 2001)**. A foreign gene can be transferred into fish *in vivo* by introducing DNA either into embryos or directly into the somatic tissues of adults **(Sudha *et al.*, 2001; Weete *et al.*, 2002; SY, 2004; Assem & El-Zaeem, 2005)**. The following techniques are often used to insert foreign DNA into embryos: gonad-mediated gene transfer, sperm-mediated gene transfer, electroporation, and microinjection **(Maclean, 1998; El-Zaeem, 2011)**. Injecting foreign DNA directly into muscle tissue is a rapid and efficient approach to introduce it, according to and **Ono *et al.* (1990)** and **Wolff *et al.* (1990)** in adult mice. Additionally, multiple studies have demonstrated gene transfer and expression after intramuscular direct injection of foreign DNA into fish skeletal muscles, suggesting a potential quick and simple method of improving fish characteristics **(Assem & El-Zaeem, 2005; El-Zaeem, 2012)**.

The current work aimed to investigate the fate of foreign DNA fragments isolated from common carp *Cyprinus carpio*, after direct intramuscular injection into African catfish *Clarias gariepinus*.

MATERIALS AND METHODS

Fish origin

The African catfish (*Clarias gariepinus*) fingerlings used in this study were obtained from Brothers hatchery, Kafr El-Sheikh, Egypt. African catfish were transported

to the Fish Production Laboratory, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University, Alexandria, Egypt.

Preparation of genomic DNA for injection

High molecular weight DNA was extracted from the frozen tissues of common carp (*Cyprinus carpio*). The DNA extraction was performed according to the manufacturer's protocol for the GeneJET Genomic DNA Purification Kit from Thermo Scientific. The quality and concentration of the DNA yield were tested using a Nanodrop spectrophotometer (BioDrop, England). The extracted DNA was digested by Eco R1 restriction enzyme type II, which cleaves DNA between guanine and adenine according to **Tsai *et al.* (1993)**.

Experimental design

Culture condition

African catfish were acclimatized to laboratory conditions for two days. Fingerlings with a body weight of 100g were divided into 3 groups and two replicates for each group, stocked at the rate of 3 fish per aquarium. The aquaria of dimensions 100 × 34 × 50 cm were supplemented with continuous aeration. All fish were fed a commercial diet with 45% protein for 10 days. Nearly half of the volume of water in the aquaria was changed daily by freshly stocked tap water, and the aquaria were cleaned every day before feeding. The aquaria were covered with black tarpaulin to block light and provide a suitable environment for the fish.

Injection of foreign DNA *in vivo*

According to **Assem and El-Zaeem (2005)** method, the DNA concentrations of 65µg/ 0.1ml/ fish (T1), 130µg/ 0.1ml/ fish (T2), and 195µg/ 0.1ml/ fish (T3) were prepared and injected into the muscles of African catfish using a hypodermic needle. All fish were anesthetized using clove oil at a concentration of 2mg/ L for 5-10 minutes before injection. The injection was applied to the three groups of African catfish fingerlings.

Sample collection

Samples were collected from the caudal fin tissues of all fish prior to the injection to serve as a control for the same fish after the injection. Then, 10 days following the injection, all fish were collected, and the fish muscles and gonads were immediately removed through dissection after being anesthetized with MS-222 (100mg/ L). All tissues were then frozen in liquid nitrogen and stored at -80°C until analysis. All animal maintenance and handling procedures adhered to the recommendations of the Institutional Animal Care Use Committee, Alexandria University, Egypt (Alex-IACUC) review report AU: 19/25/05/13/3/54.

DNA extraction:

The DNA of six fish (before injection) and six fish (after injection) from the three treatments, with one fish taken from each replicate, was extracted according to the manufacturer's protocol using the GeneJET Genomic DNA Purification Kit (K0721, Thermo Fisher).

DNA sequencing

The *glutathione reductase* gene (*gsr*) sequence was utilized as a confirmatory molecular marker for the DNA transfer technique in the muscles and gonads of African catfish (*Clarias gariepinus*). DreamTaq Green PCR Master Mix (2X) (K1081, Thermo Fisher, USA) was employed for specific gene amplification following the manufacturer's protocol using the Creacon (Holland, Inc) polymerase chain reaction (PCR) system cycler. The primer sequences for *gsr* were designed using the Primer Blast tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and are listed in Table (1). The PCR cycle was run with an initial denaturation at 95°C, annealing at 60.54°C, and extensions at 72°C.

After preparing a 1.0% agarose solution in accordance with the manufacturer's instructions, the electrophoresis apparatus was filled with the electrophoresis buffer, and the comb was taken out, resulting in 6 or 10 wells for PCR product applications when a DNA ladder (peqGOLD 1 kb DNA-Ladder, Peqlab, VWR) was present. For 100 minutes, electrodes were attached to the power supply at 80 volts. After removing the gel from its bed, it was placed on the gel staining tray and stained for 30 minutes with ethidium bromide before being destained for 20 minutes with distilled water.

Data analysis was conducted using Totallab analysis software (www.totallab.com Ver.1.0.1) and a gel documentation system (Geldoc-it, UVP, England). A 1500bp positive amplicon was eluted from an agarose gel. Micro spin filters were used to purify the resultant PCR products, and spectrophotometer quantification was performed. ABI PRISM® 3100 Genetic Analyzer (Micron-Corp Korea) was used for sequence analysis.

Table 1. Primer of *glutathione reductase* (*gsr*) gene under study

Primer sequence (5'-3')	Product length (bp)	Annealing temp. (°C)
F: GCAGTGGAGATGGCTGGTATC **R:** CCACCTCATGGATGGTGTCATACT	262	60.54°C or 61.97°C

Inter-simple sequence repeat (ISSR) technique

Inter-Simple Sequence Repeat (ISSR) was applied as a molecular fingerprinting technique via five specific primers to evaluate the genetic similarity between Injected and Non-injected samples. DreamTaq PCR Master Mix (2X) (K1071, Thermo fisher. USA)

was used as manufacturer protocol for Inter-Simple Sequence Repeat (ISSR) with specific primer (Table 2) and thermal cycler as follows: 5min at 94°C; followed by 35 cycles of 30s at 94°C, 45s at 48°C, 2min at 72°C, 7min at 72°C, and 4°C indefinitely. Total genomic DNA was amplified through GeneAmp Polymerase Chain Reaction (PCR) system cycler according to **Galbusera *et al.* (1996)**.

Table 2. Characterization of five *Clarias gariepinus* (GT) microsatellite primer sets, including locus name, repeat array, primer sequences, GenBank Accession number, specific annealing temperature, and size range of PCR products

Locus	Repeat array	Primer sequences (5'-3')	GenBank Accession number	Annealing temp. (°C)	Size range of PCR product (bp)
Cga03	$(GT)_{21}$	CACTTCTTACATTTGTGCCC ACCTGTATTGATTTCTTGCC	U30864	56	142-168
Cga05	$(GT)_{11}N_2(GT)_2$	TCCACATTAAGGACAACCACCG TTTGCAGTTCACGACTGCCG	U30866	60	204-212
Cga06	$(GT)_5$ $N_2(GT)_9$	CAGCTCGTGTTTAATTTGGC TTGTACGAGAACCGTGCCAGG	U30867	60	134-142
Cga09	$(GA)_3$ $N_3(GT)_{11}$ N $(GT)_6$ $N_2(GT)_4$	CGTCCACTTCCCCTAGAGCG CCAGCTGCATTACCATACATGG	U30871	65	180-196
Cga10	$(GT)_2$ $N_2(GT)_{15}$	GCTGTAGCAAAAATGCAGATGC TCTCCAGAGATCTAGGCTGTCC	U30870	60	102-138

The electrophoresis apparatus unit was set up at 80 volts for 100 minutes, and the PCR products were run on a 1.5% agarose gel. After that, the gel was taken out of its bed and placed on the gel staining tray to be stained for 30 minutes with ethidium bromide and then destaining for 20 minutes with distilled water.

To assign loci for each primer and estimate fragment sizes, a gel documentation system (Geldoc-it, UVP, England) was utilized. Bands were scored as diallelic for each allocated locus (1 = present; 0 = absent). NTSYSpc2.01 software was then used to determine the similarity percentages based on a presence-absence data matrix.

RESULTS

DNA sequencing

The *gsr* sequence was used as a molecular marker confirmatory method for DNA transferring technique in muscles and gonads of African catfish (*Clarias gariepinus*). The GenBank BLAST search was performed on all sequenced samples from gonads (T1g1,

T1g2, T2g1, T2g2, T3g1, and T3g2 which are accessioned in GeneBank under PV774428, PV774429, PV774430, PV774431, PV774434 and PV774435, respectively) and muscles (T1m1, T1m2, T2m1, T2m2, T3m1, and T3m2 which are accessioned in GeneBank under PV774424, PV774425, PV774426, PV774432, PV774433 and PV774427, respectively) of African catfish. These samples have been correctly identified as the *gsr* based on their sequence identity scores to sequences from *Cyprinus carpio*, which was injected into African catfish muscles, as indicated in Tables (3, 4), respectively. According to the results, all bases of the low concentration samples (T1g1, T1m1, and T1m2) exhibited a perfect 100% match with the *Cyprinus carpio gsr* gene. In contrast, the high concentration samples (T3g1 and T3g2) from gonads showed the lowest identity scores of 93.21 and 88.04%, respectively. Similarly, T3m1 (from muscles) exhibited a relatively lower identity score of 96.82%.

Table 3. Identification of *glutathione reductase* gene sequences for gonad samples of different treatments

Samples	Organism	Gene identity	Sequence ID	Identity %
T1g1	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	100.00%
T1g2	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	99.14%
T2g1	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	96.92%
T2g2	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	94.27%
T3g1	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	93.21%
T3g2	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	88.04%

*****T1g1 and T1g2 are gonad samples of the dose of 65 µg/0.1 ml/fish; T2g1 and T2g2 are gonad samples of the dose of 130 µg/0.1 ml/fish; T3g1 and T3g2 are gonad samples of the dose of 195µg/ 0.1ml/ fish.

Additionally, the Clustal Omega software was used to create MultiAlignments sequences to the gonads and muscles six sequences and compare them with the reference sequence (JN126053.1 *Cyprinus carpio grs*), resulting in 239 nucleotide sites. The alignment reveals highly conserved regions alongside regions exhibiting insertions, gaps, indels, and mismatched data. As illustrated in Fig. (1), prominent mismatched and indel motifs at nucleotide positions (106-164, 178-179, and 210-239) are noted in T3g2; also, in T3g1 there are prominent mismatched and indel motifs at nucleotide positions 132-164 and 178-179. Furthermore, Fig. (2) shows indel and mismatched motifs observed at many positions in T3m1 (1-17 and 176-181) and T3m2 (193-202 and 219-234), as well as in T2m2 (1-17, 37-51, and 209-224). These variations, along with those observed in Tables

(1, 2), may stem from the integration of the injected common carp DNA with the African catfish DNA, leading to sequence alterations in the treated samples.

Table 4. Identification of *glutathione reductase* gene sequences for muscle samples for different treatments

Samples	Organism	Gene identity	Sequence ID	Identity %
T1m1	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	100.00%
T1m2	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	100.00%
T2m1	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	96.28%
T2m2	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	97.03%
T3m1	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	96.82%
T3m2	*Cyprinus carpio*	*glutathione reductase* mRNA, partial cds	JN126053.1	98.21%

*****T1m1 and T1m2 are muscle samples of the dose of 65µg/ 0.1ml/ fish; T2m1 and T2m2 are muscle samples of the dose of 130 µg/0.1 ml/fish; T3m1 and T3m2 are muscle samples of the dose of 195µg/ 0.1ml/ fish.

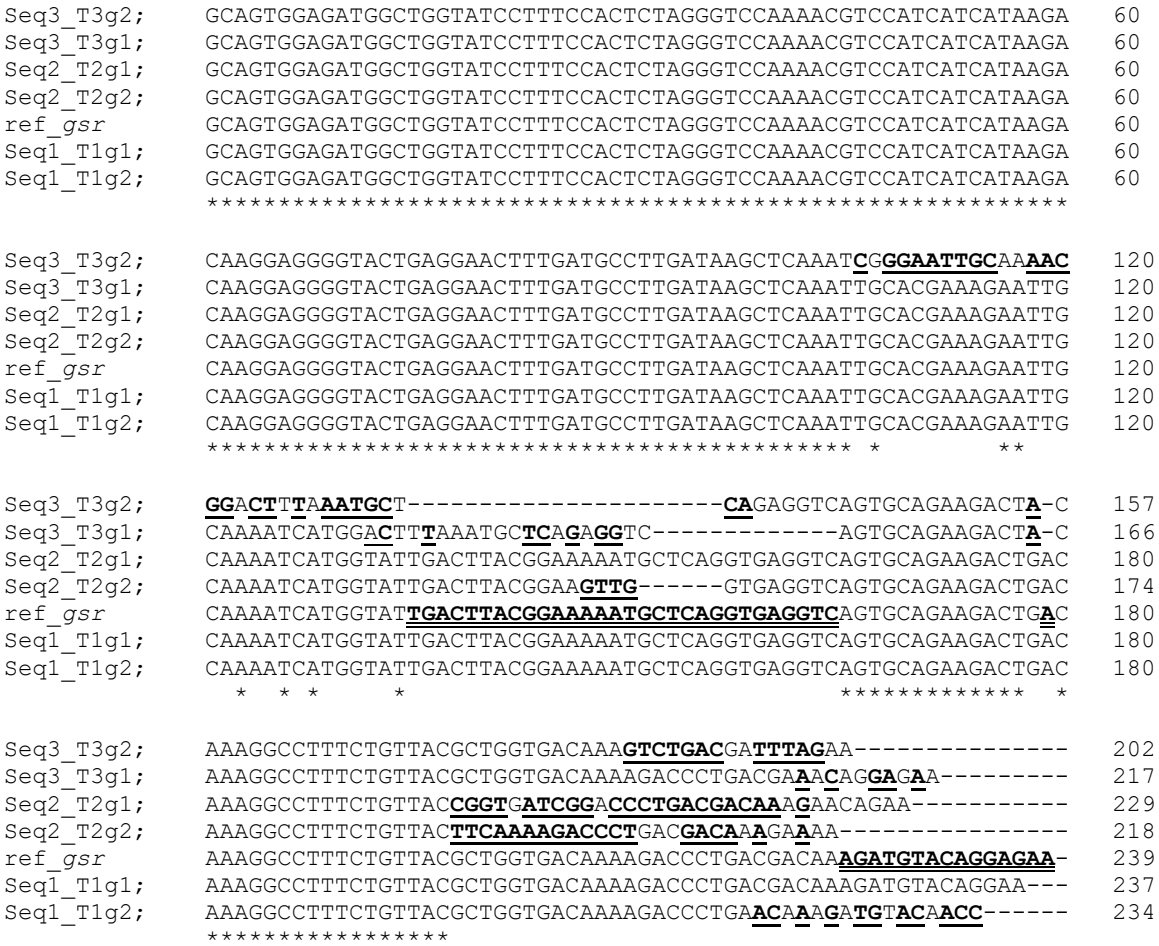

Fig. 1. DNA sequence alignment of *glutathione reductase* gene (240 bp) among the six sequenced treated samples of African catfish gonads and JN126053.1 *Cyprinus carpio glutathione reductase*. The asterisks represent similarity. The green bold sequences with underline refer to the mismatched positions, and the red bold sequences with double underline refer to the Indel positions. T1g, T2g, and T3g are gonad samples from different doses. g1 and g2 are replicates

```
Seq2_T2m2;      ------GCAGTGGAAACTATCCTTTCCACTCTAGGG-------TCCAAAAAATCATAAGA   47
Seq3_T3m2;      GCAGTGGAGATGGCTGGTATCCTTTCCACTCTAGGGTCCAAAACGTCCATCATCATAAGA   60
Seq3_T3m1;      ---GCATGAACTGGCGTTATCCTTTCCACTCTAGGGTCCAAAACGTCCATCATCATAAGA   57
Seq2_T2m1;      GCAGTGGAGATGGCTGGTATCCTTTCCACTCTAGGGTCCAAAACGTCCATCATCATAAGA   60
Seq1_T1m2;      GCAGTGGAGATGGCTGGTATCCTTTCCACTCTAGGGTCCAAAACGTCCATCATCATAAGA   60
Seq1_T1m1;      GCAGTGGAGATGGCTGGTATCCTTTCCACTCTAGGGTCCAAAACGTCCATCATCATAAGA   60
ref_gsr         GCAGTGGAGATGGCTGGTATCCTTTCCACTCTAGGGTCCAAAACGTCCATCATCATAAGA   60
                       *    ******************           *  *********

Seq2_T2m2;      CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   107
Seq3_T3m2;      CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   120
Seq3_T3m1;      CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   117
Seq2_T2m1;      CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   120
Seq1_T1m2;      CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   120
Seq1_T1m1;      CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   120
ref_gsr         CAAGGAGGGGTACTGAGGAACTTTGATGCCTTGATAAGCTCAAATTGCACGAAAGAATTG   120
                ************************************************************

Seq2_T2m2;      CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGACTGAC   167
Seq3_T3m2;      CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGACTGAC   180
Seq3_T3m1;      CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGATACA-   176
Seq2_T2m1;      CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGACTGAC   180
Seq1_T1m2;      CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGACTGAC   180
Seq1_T1m1;      CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGACTGAC   180
ref_gsr         CAAAATCATGGTATTGACTTACGGAAAAATGCTCAGGTGAGGTCAGTGCAGAAGACTGAC   180
                ************************************************   *

Seq2_T2m2;      AAAGGCCTTTCTGTTACGCTGGTGAACATTAGTCCCCCATGTACAGAC-----------   215
Seq3_T3m2;      AAAGGCCTTTCTAATCTAGTCCTGACAAAAGACCCTGAACTAGCGTGGCGGTGTGCAA-   238
Seq3_T3m1;      -AAGGCCTTTCTGTTACGCTGGTGACAAAAGACCCTGACGACAAAGATGTACAAACC--   232
Seq2_T2m1;      AAAGGCCTTTCTGTTACGCTGGTACTTAGCCCACGACAAAGATGTACAGGACA------   233
Seq1_T1m2;      AAAGGCCTTTCTGTTACGCTGGTGACAAAAGACCCTGACGAACCGGTACATTAA-----   234
Seq1_T1m1;      AAAGGCCTTTCTGTTACGCTGGTGACAAAAGACCCTGACGACAAAGAAACCAGGAGAA-   238
ref_gsr         AAAGGCCTTTCTGTTACGCTGGTGACAAAAGACCCTGACGACAAAGATGTACAGGAGAA   239
                 ***********  *    *  *    *     *
```

Fig. 2. DNA sequence alignment of *glutathione reductase* gene (239 bp) among the six sequenced treated samples of African catfish muscles and JN126053.1 *Cyprinus carpio glutathione reductase*. The asterisks represent similarity. The green bold sequences with underline refer to the mismatched positions, and the red bold sequences with double underline refer to the Indel positions. T1m, T2m, and T3m are muscle samples from different doses. m1 and m2 are replicates.

Inter-simple sequence repeat (ISSR) fingerprinting

All DNA samples from three treatments of catfish were examined using Inter-Simple Sequence Repeat (ISSR) fingerprinting. Five primers were utilized to assess DNA fingerprinting diversity across the three injection treatments of African catfish, generating a total of 509 amplicons with molecular sizes ranging from 14 to 275 bp. The highest number of amplicons was obtained with P5 (130), while P2 yielded the lowest number (67). All injected fish subjected to different concentrations of foreign DNA displayed genetic diversity after injection, as presented in Table (5). Moreover, the ISSR pattern is illustrated in Figs. (3, 4). These findings provided a very clear detection of DNA polymorphisms in the three treatments of catfish studied.

Table (6) presents data on genetic similarity coefficients among the three treatments of African catfish based on ISSR data. Specifically, the genetic similarity (GS) between fish before and after receiving an injection of 65 µg/0.1 ml/fish (T1B vs. T1A) was 0.61. For the 135 µg/0.1 ml/fish (T2B vs. T2A), the GS was 0.65. Meanwhile, for the 195 µg/0.1 ml/fish treatment (T3B vs. T3A), the GS was 0.56.

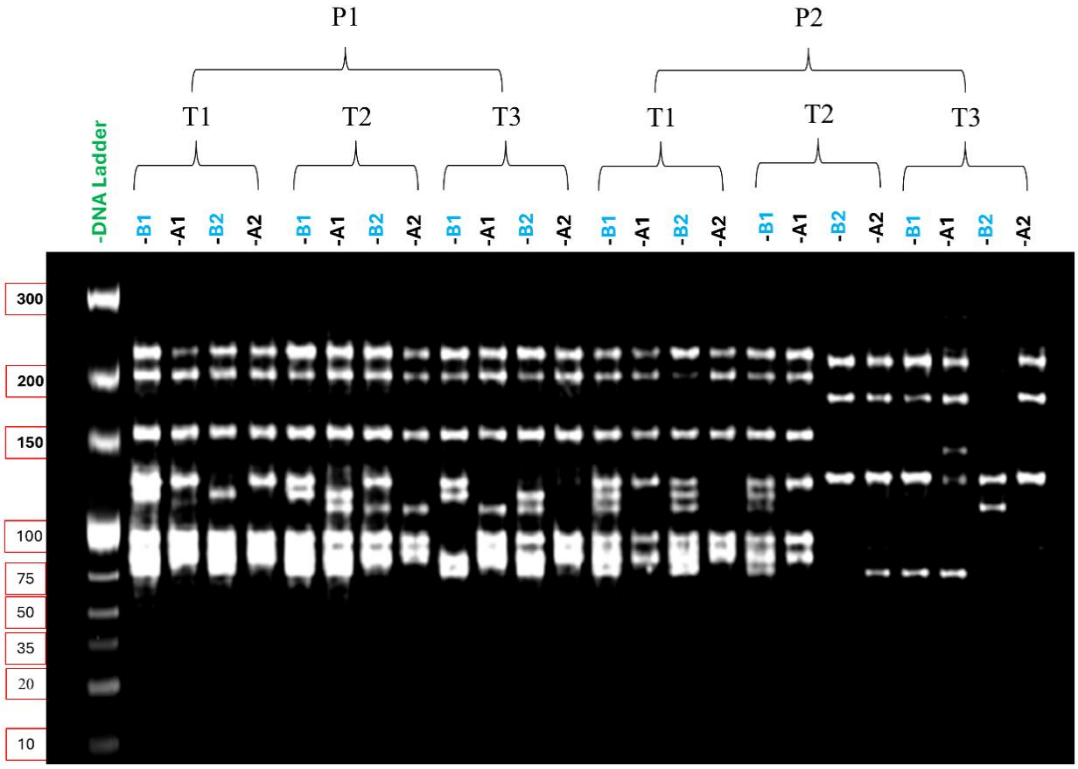

Fig. 3. Inter-Simple Sequence Repeat (ISSR) fingerprinting technique of the first two primers (P1 and P2) for three treatments T1, T2, and T3 (before and after) of injection for catfish samples

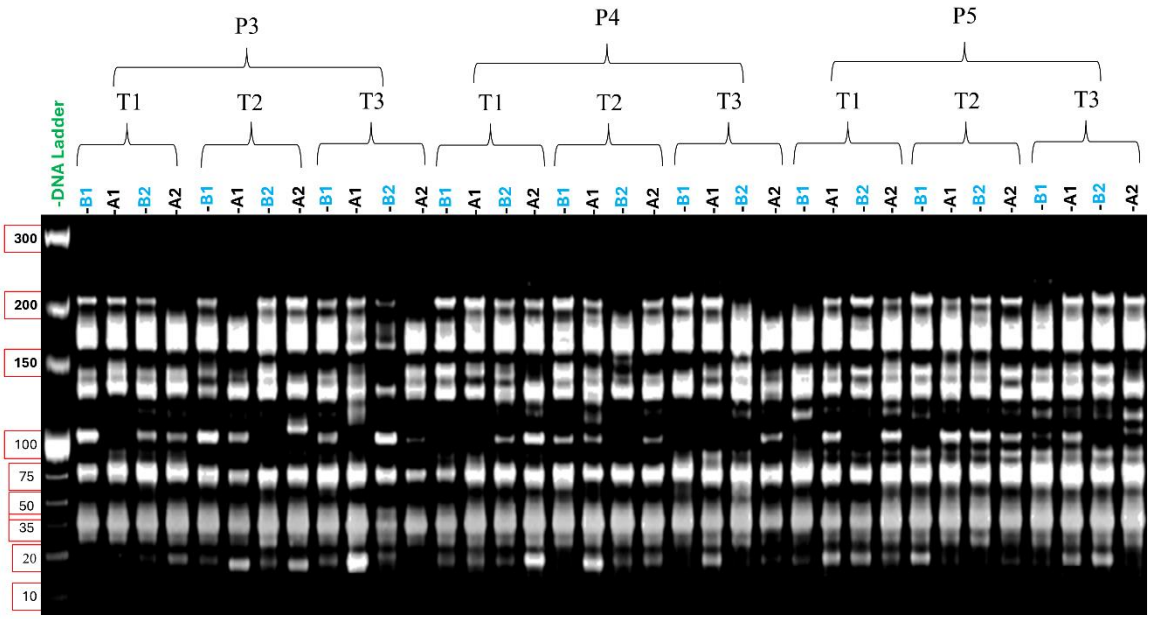

Fig. 4. Inter-Simple Sequence Repeat (ISSR) fingerprinting technique of the second three primers (P3, P4, and P5) for three treatments T1, T2, and T3 (before and after) of injection for catfish samples

Table 5. Amplicon size (bp), total no. of Amplicons, and Polymorphism among the studied African catfish under different treatments of injection according to different ISSR primers used

ISSR Primers	Amplicon Size (bp) Range (bp)	Total No. of Amplicons	Total No. of Polymorphic Amplicons	Polymorphism (%)											
				T1				T2				T3			
				Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
				R1	R1	R2	R2	R1	R1	R2	R2	R1	R1	R2	R2
P1	80-232	87	27	37.5	28.6	42.9	33.3	12.5	30.0	28.6	16.7	42.9	33.3	37.5	28.6
P2	77-275	67	19	33.3	0.0	55.6	20.0	33.3	0.0	0.0	25.0	0.0	42.9	50.0	66.7
P3	14-210	104	26	12.5	22.2	30.0	12.5	12.5	22.2	50.0	37.5	30.0	12.5	25.0	25.0
P4	14-229	121	29	20.0	20.0	10.0	30.8	27.3	20.0	22.2	22.2	33.3	45.5	20.0	11.1
P5	14-231	130	22	0.0	36.4	10.0	18.2	0.0	8.3	18.2	10.0	16.7	16.7	20.0	38.5
Mean or Total	--	509	123	20.66	21.44	29.70	22.96	17.12	18.08	23.8	21.5	24.58	30.18	30.50	33.98

*P1-P5 are primers, and R1and R2 are replicates.

Table 6. Jaccard's similarity coefficients among three treatments of African catfish based on ISSR data of all primers used

Treatments	**T1B**	T1A	**T2B**	T2A	**T3B**	T3A
T1B	1.00	-	-	-	-	-
T1A	**0.61**	1.00	-	-	-	-
T2B	-	-	1.00	-	-	-
T2A	-	-	**0.65**	1.00	-	-
T3B	-	-	-	-	1.00	-
T3A	-	-	-	-	**0.56**	1.00

*T1B, T2B and T3B are treatments before injection; while, T1A, T2A and T3A are treatments after injection.

DISCUSSION

Indirect methods of transferring new genetic material into tissues have been the mainstay of postnatal gene therapy attempts; target cells are extracted from the body, infected with viral vectors that contain the new genetic information, and then reintroduced into the body. Direct gene delivery into tissues *in vivo* without the use of viral vectors would be beneficial for certain applications **(Wolff *et al.*, 1990)**. In this topic, **Ali (2001)** stated that it is possible to isolate the entire gene, as well as its introns and exons, and then transfer them to the target host. With mRNA and reverse transcriptase treatments, this method focuses on using the entire gene, including introns and exons, rather than just the exons. Since introns, which function as retrotransposons, allow foreign genes to be introduced into cells, there is no need to use any type of virus.

In this study, the DNA sequence identity score results for the *gsr* sequences from *Cyprinus carpio* after intramuscular injection into African catfish revealed a clear correlation with the concentration of injected DNA. Specifically, Tr3g1 and Tr3g2 exhibited the lowest identity score among gonad samples, while Tr3m1 showed a relatively lower identity score among muscle samples. Generally, increasing the concentration of foreign DNA injected into fish muscles resulted in a decrease in identification percentage when compared to the *Cyprinus carpio* reference sequence. Moreover, the results of the DNA sequence alignment of the *gsr* gene demonstrated the presence of mismatched and indel sites across various fish muscle and gonad samples that were exposed to various amounts of foreign DNA, indicating that the variations were precisely identifiable and are consistent with the integration of the injected *Cyprinus carpio* DNA into the African catfish genome.

According to the findings of **Hansen *et al.* (1991)**, the quantity of injected DNA determines the level of foreign gene expression. Additionally, he stated that 50µg of DNA was roughly the ideal amount, as opposed to 100µg, which didn't increase the level of CAT activity. Additionally, **Rahman and Maclean (1992)** observed that an increase in the injection volume of DNA solution didn't appear to increase CAT activity. This could be because high amounts of foreign DNA induce nuclease activity to destroy the transgene copies.

Furthermore, the genetic polymorphism analysis using the ISSR technique before and after the injection of foreign DNA fragments into catfish muscle showed varying values in fish injected with DNA compared to the same fish before injection. Furthermore, all injected fish under different concentrations of foreign DNA showed genetic diversity after injection. Additionally, the interpopulation genetic similarity indicated that the lowest genetic similarity was observed between fish in the Tr3A and Tr3B treatments. All results revealed the presence of differences in Tr3, which are likely due to the integration of injected common carp DNA into the catfish muscles. It may be due to the differences in the DNA molecule between the same fish before and after injection with a foreign DNA fragment, as a result of the direct injection of different concentrations of common carp DNA. Moreover, some fragments of common carp DNA may be randomly integrated into *C. gariepinus* muscle genome.

The ISSR approach was used because to its simplicity and dependability, as well as its abundance of polymorphism and highly reproducible results, in evaluating the molecular genetic variability within and among several living organisms **(Kol & Lazebny, 2006; Lalhruaitluanga & Prasad, 2009)**. **Liu *et al.* (2006)** used ISSR analysis, which has been shown to be a sensitive and reproducible method for studying fish population genetics, to examine genetic diversity in three *Paralichthy solivaceus* populations. Additionally, **Saad *et al.* (2012)** reported the genetic diversity among some tilapia species based on ISSR Markers. According to **El-Zaeem *et al.* (2018)**, genotype analysis based on ISSR fingerprinting can be utilized to differentiate across populations of common carp (*Cyprinus carpio*) with similar results at the intraspecific level.

Additionally, fragments of foreign DNA may have been randomly integrated into the genomes of catfish. These results correspond with findings from **El-Zaeem (2012)**, which showed a higher percentage of genetic polymorphism among grey mullet that received foreign DNA compared to their control group.

Sudha *et al.* (2001) stated that the expression of intramuscular injection of foreign DNA is evident in several non-muscle tissues of fish, such as skin, epithelia, pigment cells, blood vessel cells, and neuron-like cells and as mentioned in an article by **Hallerman *et al.* (2022)**, restriction enzymes cause particular DNA breaks that are then repaired by the

organism's DNA repair systems using either homology-directed or non-homologous end-joining techniques. This may result in a gene knockout, a point mutation or deletion, or the introduction of a new DNA sequence, depending on the template DNA that was supplied. These results are consistent with our findings that indicate all sequenced samples from the gonads and muscles of African catfish have been correctly identified and have high sequence similarity scores to sequences of the *gsr* gene from *Cyprinus carpio* DNA injected into African catfish muscles. Additionally, the results of the MultiAlignments sequencing revealed nucleotide sites with alignment gaps, indels, missing and mismatching data in all treatments.

In this study, the duration of observation for the foreign DNA fate was limited to 10 days post-injection. While this period allowed us to observe strong initial expression, it does not provide insights into the long-term persistence or stability of the expressed genes in *Clarias gariepinus*. Future studies could benefit from extended observation periods to assess the long-term fate of the foreign DNA.

CONCLUSION

In conclusion, this study provides preliminary insights into the direct intramuscular injection of foreign DNA that would be an efficient, time- and cost-effective method for transferring DNA fragments between organisms to create new genetic lines. Thus, this approach may be used in subsequent research to enhance fish traits and the possibility of functional studies of various tissues, DNA vaccine delivery, and muscular expression of other beneficial genes.

REFERENCES

Aleström, P. (2001). Genetically modified fish in future Aquaculture: Technical, environmental and management considerations. International Service for National Agricultural Research.

Ali, A. M. M. (2001). The role of genomic DNA and introns in gene transfer. *I*nternational Journal of Biotechnology, *3*(3-4), 411-418.

Assem, S. S. and El-Zaeem, S. Y. (2005). Application of biotechnology in fish breeding. II: production of highly immune genetically modified redbelly tilapia, Tilapia zillii. African Journal of Biotechnology, 4(5).

El-Zaeem, S. (2001). Breeding studies in Tilapia. Faculty of Agriculture (Saba-Basha), Alexandria University, Egypt.

El-Zaeem, S.; El-Dahhar, A.; Fernandes, J. and Shyaa, A. (2018). Phenotype Variation and Phyolgeny of Different Population of Striped Red Mullet (*Mullus surmuletus*, Linnaeus 1758). Journal of the Arabian Aquaculture Society, 13(1).

El-Zaeem, S. Y. (2011). Growth comparison of Nile tilapia (*Oreochromis niloticus*) and Blue tilapia,(*Oreochromis aureus*) as affected by classical and modern breeding methods. African Journal of Biotechnology, 10(56), 12071-12078.

El-Zaeem, S. Y. (2012). Extraordinary Mullet growth through direct injection of foreign DNA. African Journal of Biotechnology, 11(33), 8295-8301.

Galbusera, P.; Volckaert, F. A.; Hellemans, B. and Ollevier, F. (1996). Isolation and characterization of microsatellite markers in the African catfish *Clarias gariepinus* (Burchell, 1822). Mol Ecol, 5(5), 703-705. https://doi.org/10.1111/j.1365-294x.1996.tb00366.x

Hallerman, E. M.; Dunham, R.; Houston, R. D.; Walton, M.; Wargelius, A. and Wray-Cahen, D. (2022). Towards production of genome-edited aquaculture species. Reviews in Aquaculture, 15(2), 404-408. https://doi.org/10.1111/raq.12739

Hansen, E.; Fernandes, K.; Goldspink, G.; Butterworth, P.; Umeda, P. K. and Chang, K.-C. (1991). Strong expression of foreign genes following direct injection into fish muscle. FEBS letters, 290 (1-2), 73-76.

Hendy, A. M. (2019). Phylogenetic Differentiation Among Some Different African Catfish (*Clarias Gariepinus*) Population. MSc thesis. Faculty of Agriculture Saba Basha. Alexandria University, Egypt. http://srv3.eulc.edu.eg/eulc_v5/Libraries/Thesis/BrowseThesisPages.aspx?fn=PublicDrawThesis&BibID=12865160

Kol, N. and Lazebny, O. (2006). Polymorphism of ISSR-PCR markers in Tuvinian population of reindeer Rangifer tarandus L. Russian Journal of Genetics, 42, 1464-1466.

Lalhruaitluanga, H. and Prasad, M. (2009). Comparative results of RAPD and ISSR markers for genetic diversity assessment in Melocanna baccifera Roxb. growing in Mizoram State of India. African Journal of Biotechnology, 8(22).

Levy, J.; Marins, L. and Sanchez, A. (2000). Gene transfer technology in aquaculture. Hydrobiologia, 420, 91-94.

Liu, Y.-G.; Chen, S.-L.; Li, J. and Li, B.-F. (2006). Genetic diversity in three Japanese flounder (*Paralichthys olivaceus*) populations revealed by ISSR markers. Aquaculture, 255(1-4), 565-572.

Maclean, N. (1998). Regulation and exploitation of transgenes in fish. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 399(2), 255-266.

Maclean, N. and Laight, R. J. (2000). Transgenic fish: an evaluation of benefits and risks. Fish and Fisheries, 1(2), 146-172.

Melamed, P.; Gong, Z.; Fletcher, G. and Hew, C. L. (2002). The potential impact of modern biotechnology on fish aquaculture. Aquaculture, 204(3-4), 255-269.

Ono, T.; Fujino, Y.; Tsuchiya, T. and Tsuda, M. (1990). Plasmid DNAs directly injected into mouse brain with lipofectin can be incorporated and expressed by brain cells. Neuroscience letters, 117(3), 259-263.

Oyeleye, O.; Ola, S. and Omitogun, O. (2016). Ovulation induced in African catfish (*Clarias gariepinus*, Burchell 1822) by hormones produced in the primary culture of pituitary cells. International Journal of Fisheries and Aquaculture, 8(7), 67-73.

Rahman, A. and Maclean, N. (1992). Fish transgene expression by direct injection into fish muscle. Molecular Marine Biology and Biotechnology, 1(4-5), 286-289.

Saad, Y.; Rashed, M.; Atta, A. and Ahmed, N. (2012). Genetic diversity among some tilapia species based on ISSR markers. Life Science Journal, 9 (4), 4841-4846.

SARMAŞIK, A. (2003)**.** Application of gene transfer technology for genetic improvement of fish. Turkish Journal of Zoology, 27(1), 1-6.

Shourbela, R.; Tohamy, H. and El-Hawarry, W. (2020). Induced spawning of African catfish (*Clarias gariepinus* Burchell 1822) after pre-spawning prophylactic disinfection; the breeding performance and tissue histopathological alterations are under scope. Iranian Journal of Fisheries Sciences, 19(1), 309-324.

Sudha, P. M.; Low, S.; Kwang, J. and Gong, Z. (2001). Multiple tissue transformation in adult zebrafish by gene gun bombardment and muscular injection of naked DNA. Marine biotechnology, 3, 119-125.

SY, E.-Z. (2004). Alteration of the productive performance characterestics of *Oreochromis niloticus* and *Tilapia zillii* under the effect of foreign DNA injection. Egypt. J. Aquat. Biol. Fish, 8(1), 261-278.

Tsai, H.; Tseng, C.-F. and Kuo, T.-T. (1993). Expression of rainbow trout growth hormone cDNA in yeast. Bull Inst Zool Acad Sin, 32, 162-170.

Tsai HuaiJen, T. H. (2003). Transgenic fish: researches and applications.

Wang, Y.; Hamid, N.; Jia, P. P. and Pei, D. S. (2021). A comprehensive review on genetically modified fish: key techniques, applications and future prospects. Reviews in Aquaculture, 13(3), 1635-1660.

Weete, J.; Cummins, K. and Lovell, R. (2002). Effect of Rainbow Trout Growth Hormone Complementary DNA on Body Shape, Carcass Yield, and Carcass Composition of F and F2 Transgenic Common Carp (*Cyprinus carpio*). Mar. Biotechnol, 4, 604-611.

Wolff, J. A.; Malone, R. W.; Williams, P.; Chong, W.; Acsadi, G.; Jani, A. and Felgner, P. L. (1990). Direct gene transfer into mouse muscle *in vivo*. Science, 247(4949), 1465-1468.