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DNA Fingerprints of Two Tilapia Fish Species of Euphrates River at Governorate of Al-Muthanna Using RAPD Markers

Abstract- *During the last decade, tilapia species (Cichlidae: Teleostei) became members of Iraqi fish fauna. They characterized with morphological similarity among genera and species. That makes species differentiation not easily. Molecular methods followed to differentiate between redbelly tilapia Coptodon zillii (Gervais, 1848) and blue tilapia Oreochromis aureus (Steindachner, 1864) of the Euphrates River sector at Al-Samawah city. RAPD-PCR method used to create the genetic fingerprints of two tilapia fish species. Seven decamer primers (OPA08, OPA10, OPA13, OPA17, OPA19, OPB08 and OPC02) used to amplify DNA fragments using PCR-RAPD technique. Forty-four bands scored after electrophoresis on 2% agarose gel along with molecular marker fragmented to each 100 base pair. The molecular weight of bands was calculated using PhotoCapt-MW software. The volume of bands ranged from C. zillii 168 bp to 2227 bp while they ranged from 62 bp to 2154 bp in O. aureus. The results achieve the RAPD fingerprints of two tilapia species in Euphrates River at for genetic Al-Samawa city and draw the genetic tree with the same species from Shatt A-Arab River in Basrah city. The study concluded that there is the closest relatedness among tilapia populations from Euphrates and Shatt Al-Arab Rivers. The results proved that RAPD markers were efficient to generate DNA fingerprints of tilapia fish species. Furthermore, the utilizing of the RAPD markers can differentiate the two studied species. The present study may be the first genetic study on these tilapia fish species. Moreover, this would be the baseline studies in the future. In addition, this study would be valuable for conservation program and documentation of identities of tilapia fish species in Iraqi inland waters.*

Keywords- *DNA, Fingerprints, PCR, RAPD, Tilapia, Iraq, Euphrates*.

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1. Introduction

In spite of scientist objection to introducing tilapia fish for culturing in Iraqi aquatic environment, they reached it in an unknown way. They invested various habitats of Iraqi waters including freshwater of rivers, lakes, marshes and brackish waters [1]. The tilapia fish species belong to family Cichlidae. They distributed via various environments of Iraqi inland waters. The first time Coptodon zillii (Gervais, 1848) recorded in Euphrates River near Al-Musaib town of governorate Babylon by Saleh [2]. While three species Coptodon zillii, Oreochromis aureus (Steindachner, 1864) recorded by Mutlak and Al-Faisal [3] and Oreochromis niloticus by Al-Faisal and Mutlak [4] in Shatt Al-Arab River. Tilapia species distribute across tropical and subtropical of Africa and south-west Asia [5]. Some tilapia species play an important role in aquaculture activities of African countries according to the Food and Agriculture Organization in United Nation report [6]. Investing of Iraqi waters make competition between tilapia with the native species. They compete on niches and nutrition resources. In addition to their omnivorous nutrition, this led them to feed on the oval, larval stages and the aquatic plant, which represents the nesting component of native species [7]. The morphological similarity among tilapia species makes the species differentiation using morphological characteristics complicated except the fish taxonomists. So electrophoresis of proteins utilized to distinguish among tilapia species [8]. This protein method failed to discriminate among Oreochromis niloticus subspecies [9]. Recently, genetic markers of Deoxyribonucleic acid (DNA) amplification by polymerase chain reaction technique (PCR) particularly mitochondrial DNA successfully used to differentiate among O. niloticus following

the Restricted Fragment Length Polymorphism DNA method (RFLP) which needs to restriction enzymes after DNA fragment amplification [10]. The RFLP method needs previous knowledge about the gene sequence and restriction sites and restriction enzymes that create informative bands in order to check the DNA polymorphism [11].

Now a day, Random Amplified Polymorphism DNA (RAPD) method utilizes many oligonucleotides often decamer sequence to amplify unknown number, volume and sequence DNA bands [12, 13]. The RAPD pattern that amplified would be specific to the species, subspecies, or population. The RAPD method is capable of detecting the genetic variation among the species, without previous information of gene sequence [14]. RAPD bands pattern appearing via electrophoresis on the agarose gel would be a DNA fingerprint of the mention species or population. RAPD method frequently used to detect DNA fingerprint of fish species. So many international scientists used RAPD markers to differentiate among tilapia species and subspecies [15, 16]. While Ahmed et al. [17] studied tilapia species on the genus and species levels in Egypt inland waters. In the same time, the RAPD method followed to create genetic fingerprints of many other fish species; Callejas and Ochando [18] utilized RAPD markers to differentiate among Barbus species in Spanish rivers and some cyprinid fish species in of Iberian peninsula inland waters [19, 20] while it used to distinguish among Stripped Red Mullet populations in various environments [21]. Whereas Yoon and Kim [22]. were studied the genetic variation among cultured Silurus asotus in Korea. On the same side, Jini [23] used RAPD method to discriminate among Etroplus maculatus populations .

Locally, RAPD genetic markers utilized to differentiate among eight cyprinid fish species of Iraqi inland waters [24]. While the same technique followed to discriminate among six Barbus species [25]. On the other side, RAPD used to discriminate among four populations of Luciobarbus xanthopterus in various Iraqi freshwaters [26]. In addition, a study on carangid species in Iraqi marine waters using the same protocol [27].

For the ecological and economic importance of tilapia species in Iraqi aquatic ecosystems, in addition, there is no genetic study on tilapia in Euphrates River of Iraq, the study aimed to differentiate between them genetically using DNA fingerprints and analyze the genetic relatedness with the tilapia population in Shatt Al-Arab River at governorate of Basrah.

2. Materials and Methods

Thirty specimens of tilapia fish collected from Euphrates River at Al-Samawah city sector in Al-Muthanna governorate and transfer to the lab by a cool box filled with ice. They classified primarily following the morphological characteristics [28]. Piece of caudal fin cut and preserved in 95% ethanol vile until extraction time. Genomic DNA extracted by Geneaid Co. Kit. The manufacturer protocol followed. DNA integration was tested by electrophoresis on 0.8% agarose gel with 70 Volt for 30 minutes. Extraction product on the agarose gel tested on UV light plate. Genomic DNA preserved in 1.5 ml under -20° C until PCR experiments.

RAPD-PCR followed using Mastermix from Bioneer Co. plus seven RAPD primers listed in the Table 1. Thermocycler programmed as in Table 2.

Table 1: Primers used in RAPD-PCR experiments of Tilapia spp

No	Primer name	symbol	Seq.	GC%
1	OPAO8	P1	GTGACGTAGG	60
2	OPA10	P2	GTGATCGCAG	60
3	OPA13	P3	CAGCACCCAC	70
4	OPA17	P4	GACCGCTTGT	60
5	OPA19	P5	CAAACGTCGG	60
6	OPB08	P6	GTCCACACGG	70
7	OPC02	P7	GTGAGGCGTC	70

Table 2: Program used for RAPD-PCR technique

Stage	Stage name	Step	Temp. (C)	Time (Min)	Cycle number
1	Initial denaturation	1	95	5	1
	Denaturation	1	95	1	35
2	Annealing	2	36	1	
	Elongation	3	72	1	
3	Final elongation	1	72	6	1

PCR products electrophoresed by 70 V for 50 minutes along with molecular marker fragmented for each 100 bp from Bioneer Co. on an agarose gel, working solution was 0.5% Tris-Boric Acid-EDTA (TBA) stained with ethidium bromide dye. Agarose gel investigated under UV light and photographed with Galaxy mobile Camera. PhotoCapt-MW software used to measure the molecular weight of amplified bands created by electrophoresis. Microsoft Office Excel used to draw the Histogram represents the profile of

RAPD product. Dendrogram tree was created by UPGMA online [29].

3. Results and Discussion

The Results of molecular analysis for RAPD-PCR products for two tilapia fish species C. zillii and O. aureus from the Euphrates River at Al-Samawah city in the governorate of Al-Muthanna show a genetic variation between two species. In addition, both species responded to all seven primers, as showed in Figures 1 and 3. In the same time, DNA fingerprints of molecular markers amplified by this reaction created various number and volume of DNA bands as showed in Figures 2 and 4.

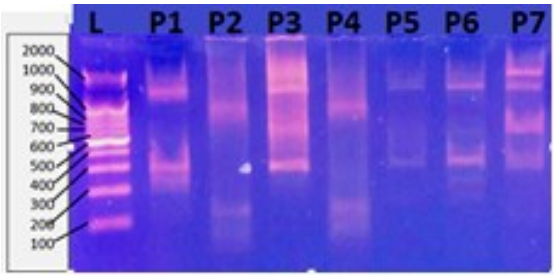

Figure 1: DNA fingerprint of tilapia fish Coptodon zillii from Euphrates River in the governorate of Al-Muthanna electrophoresed on %2 on an agarose gel, 70 V. L= Ladder, and P= Primer

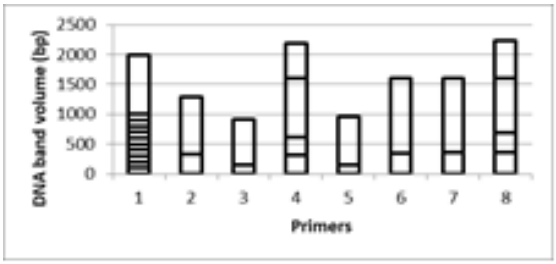

Figure 2: Histogram represents the DNA fingerprint of tilapia Coptodon zillii from the Euphrates River in the governorate of Al-Muthanna

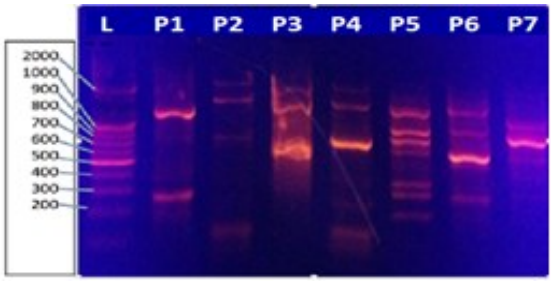

Figure 3: DNA fingerprint of tilapia fish species Oreochromis aureus from Euphrates River in the governorate of Al-Muthanna electrophoresed on %2 on an agarose gel, 70 V. L= Ladder, and P= Primer

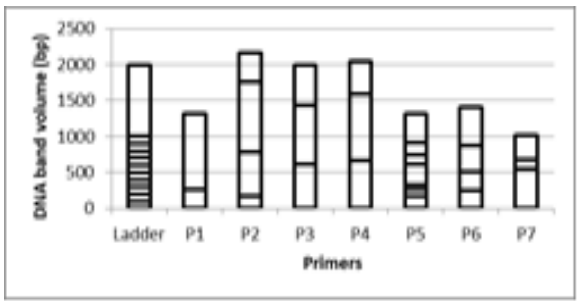

Figure 4: Histogram represents the DNA fingerprint of tilapia Oreochromis aureus from the Euphrates River in the governorate of Al-Muthanna

The DNA bands scored in tilapia C. zillii and O. aureus using seven primers were 44 bands while the faint and non-informative bands ignored. They distributed to 18 bands in C. zillii and 26 bands in O. aureus as showed in Figures 2 and 4. The band volume has ranged from 168 bp amplified by P2 to 2227 bp created by P7, both in C. zillii profile. The results in Figures 2 and 4 revealed that the primer P1 amplified four bands in both species, while the primer P5 created nine bands in both as showed in Figures 2 and 4. According to RAPD pattern, there is genetic variation between two species. The RAPD markers method was efficient to discriminate the two-tilapia species; C. zillii and O. aureus, as shown in Figures 1 and 3 .

To reveal the genetic variation and relatedness among the population of tilapia species cached of Euphrates River in the governorate of Al-Muthanna with the tilapia species cached of Shatt Al-Arab River studied by Faddagh et al. [30]. The UPGMA dendrogram of Euphrates tilapia species and Shatt Al-Arab River tilapia species drew using UPGMA online, as shown in Figure 5. Each species clustered with the same one of the different habitat. While the Oreochromis niloticus stay single branch between them.

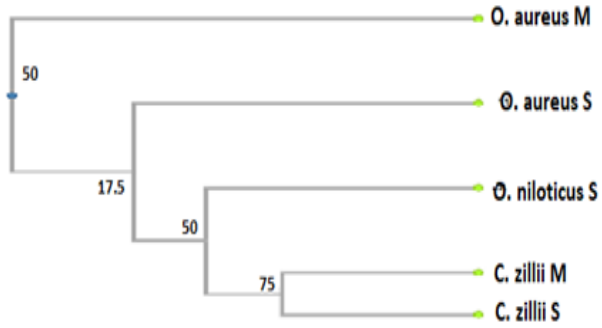

Figure 5: UPGMA dendrogram of the genetic relationship between two tilapia species of Euphrates in the governorate of Al-Muthanna (M) and three species of Shatt Al-Arab River in the governorate of Basrah (S)

Ecological isolation, water quality, temperatures, bottom properties and nutrient components included with the fish diets makes the tilapia populations categorized into two varied phenotypes. Because of that ecological diversity and environment isolation, the morphological characteristics not useful to classify the fish clearly [31]. The genetic markers method succeeded to differentiate among species and populations more than allozymes, which failed in differentiation among Barbus species [32].

Actually, using the RAPD genetic markers was beneficial in creating specific genetic fingerprint to distinguish among species of the tilapia. This results also reported by Shair et al. [33] who studied three cultured tilapia species in Saudi Arabia using single RAPD primer for differentiation among the three species and creating genetic fingerprints to each one which can be considered specific for them. While the variation in bands number and volume indicate to the genetic distance among the studied fish species and the presence of the same bands in more related species, explain the evolutionary relationships among fish species [34].

4. Conclusion

DNA fingerprints of two species revealed genetic variation between them. In addition, RAPD markers can distinguish the genetic variation, among populations, according to geographic isolation. Therefore, studying the genetic relationships among Iraqi fishes on the species and population levels using RAPD markers is capable of investigating the genetic diversity among the fish species in Iraqi waters. Therefore, using Random Amplified Polymorphic DNA (RAPD) to create DNA fingerprint gave applicable results. In the same time, the RAPD method was easy, efficient and inexpensive in comparison with other methods.

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