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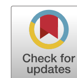
Original article

Genetic diversity in tilapia populations in a freshwater reservoir assayed by randomly amplified polymorphic DNA markers



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ABSTRACT

Genetic variation in fish stocks decreasing due to water pollution in the freshwater rivers, streams and canals. The objective of this study was to determine the genetic diversity and polymorphism in *Oreochromis niloticus* collected from the Wadi Hanefah Riyadh, Saudi Arabia by using RAPD-PCR. Total thirty fish specimens were harvested from each of four pre-determined locations of the reservoir which were designated as H1, H2, H3, and H4. Five random decamer primers were used to assess the diversity in the stock of *O. niloticus.* In this fish stock 48 bands were polymorphic and 12 were monomorphic. The maximum polymorphism (100%) was recorded in the fish samples procured from H4, followed by 88.75, 87.33 and 76.12% of the tilapia collected from H3, H2, and H4, respectively. Nei's genetic distance value was ranged as 0.0005 to 0.1006. Maximum and minimum genetic distance was recorded as 0.1006 and 0.005 in tilapia harvested from H1 and H2 locations. Average heterozygosity was ranged from 0.3009 to 0.3744. This information about the genetic polymorphism of *O. niloticus* may be used by the concerned authorities to evolve strategies to conserve the diversity of tilapia in the country.

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1. Introduction

The tilapia (*Oreochromis niloticus*) is widely cultured across the globe (Gottschalk et al., 2015). *O. niloticus* is categorized as a member of Cyprinidae (Nelson, 1994). The tilapia is cultured in every part of the world. Tilapia feeds on planktonic life to fulfill their metabolic requirements (Shair et al., 2011). The population of tilapia (*O. niloticus*) is gradually decreasing in water reservoirs due to anthropogenic activities, and water pollution and habitat degradation (Abder-Kader et al., 2013).

Genetic variation is necessary for natural stock of fish for the evolution purpose and to ensure their availability for the future generation (Xia et al., 2014, 2015). The decrease in genetic diversity in wild stock of freshwater fish species may influence on the adjustment of the fish into their changing environment (Arif and Khan, 2009; Mkare et al., 2017). The evolutionary variations are caused due to spontaneous mutation, migration, and genetic drift in the freshwater fish (Zhao et al., 2011). Genetic variation helps the s fish species to adjust in changing environment which is important for their survival. The information about the genetic structure is required to conserve the natural stocks (Carlson et al., 2015; Chauhan et al., 2007). The conservation of allelic variation is necessary for maintaining the genetic integrity and to conserve the natural stocks of freshwater fish (Perrier et al., 2011). The continuous monitoring of fish stocks in freshwater reservoirs is necessary to overcome their genetic decline (Alam et al., 2009; Islam et al., 2005). The information related about the diversity in natural stocks of fish is required to plan stocking program to observe the variation in genotype frequencies (Chupania et al., 2006; Frankham, 2010). Fisheries scientists are using different biotechnological techniques to explain the genetic diversity in fish culture (Okumus and Çiftci, 2003; Yousefian et al., 2011). DNA markers are the most commonly used tool for the conservation of fish stocks at a minimum level (Carlson et al., 2015).

The "randomly amplified polymorphic DNA (RAPD) technique is used for the investigation of genetic parameters, both in wild and inland fish stocks" (Alam and Islam, 2005). "Different biomarkers such as Microsatellites, RAPDs, AFLPs, RFLPs used to estimate the

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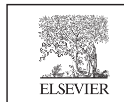

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genetic variation to plan strategies for the conservation of fish population, sex determination, identification of disease carriers, and transgenesis (Figueras et al., 2016; Basavaraju et al., 2014)". RAPDs is also in practice to determine the effect of contamination on genetic content (Liua and Cordes, 2004). The aim of this research work was to assess the genetic diversity and polymorphism in the tilapia stocks Wadi Haneffah, Riyadh, Saudi Arabia through RAPD analysis, and also to propose strategy for conservation of tilapia stocks in the freshwater reservoirs.

2. Materials and methods

2.1. Study area

"Wadi Hanefah is also known as Riyadh River/Riyadh Lake and has a length of 120 km (75 mi) from northwest to southeast, cutting through the city of Riyadh, Saudi Arabia. Riyadh City has a population of approximately 4 million. This water reservoir receives water treated by the Riyadh municipality's sewerage system and untreated discharge from local industry and adjacent areas along the length of the river. The water of this reservoir is used for irrigation of various fruit farms and vegetables grown in adjacent areas" (Mahboob et al., 2014).

2.2. Sampling of fish

Oreochromis niloticus samples (120) was collected from four different sampling locations viz., "Wadi Labn, Wadi Ubayr, Wadi Liha, and Al-Hair", which were designated as H1, H2, H3, and H4 in the Wadi Hanefah, Riyadh Saudi Arabia. Blood was collected from each fish and was stored in 95% ethyl alcohol at −20 °C. These samples were shifted to the Lab for extraction of DNA.

2.3. Isolation of DNA

Genomic DNA was isolated by the methodology of by Sambrook et al. (1989) with slight changes as explained by Yue and Orban (2005).

2.4. PCR amplification of RAPD loci

"Five random decamer primers (OPA-02, OPA-04, OPA-05, OPA-08, and OPA-09) purchased from Operon Technologies" were used in the estimation of polymorphisms. "Extracted genomic DNA was amplified by PCR" (Table 1). "The sequences of the primers were taken from the literature (Chandra et al., 2010) and oligonucleotides were custom synthesized by Eurofins genomics, Canada. Specific parental band profiles were generated by these five primers in at least three replicate PCRs. For non-denatured gel electrophoresis, 40% acrylamide gel was used".

2.5. Similarity analysis

"RAPD-ISSR data was used to generate a similarity matrix using the" Nei and Lei (1979) method.

2.6. Data analysis

"The genotypic data obtained from band counting were analyzed using the programs POPGENE and TFPGA. The genotype data for each locus were subjected to accurate analysis to estimate genetic diversity in the stock of *O. niloticus*. The banding patterns generated by RAPD markers were scored on the basis of presence or absence of visible, clear, and reproducible bands. The presence of the band was scored 1 and the absence of the band was scored 0. The RAPD loci were utilized for determination of genetic diversity, the number of polymorphic loci, and genetic distance. They were also used to construct an unweighted pair group method for the arithmetic mean for the UPGMA dendrogram for the population using Nei's unbiased distance" (Ambak et al., 2006).

For every sample, "the proportion of polymorphic loci (P %), as well as the meaning of genetic diversity (H %) was calculated by POPGENE v.1.31. POPGENE software was utilized for analysis of polymorphic loci, genetic diversity within the population, genetic diversity between populations, and construction of a dendrogram based on Nei's unbiased genetic distances. Genetic distances were estimated by utilizing TFPGA (Tool for population genetic analysis").

3. Results

3.1. RAPD analysis

In this study 48 bands were polymorphic and 12 bands were monomorphic (Table 2) from four sampling sites. The highest bands were observed in the tilapia harvested from H4 location produced by primer OPA-04 and the minimum (10 bands) in *O. niloticus* procured from H1 by primer OPA-02.

3.2. Polymorphism among the primers

The polymorphism % in the *O. niloticus* was recorded as: OPA-05 (99.9%) > OPP-08 (88.75%) > OPA-02 (85.10%) > OPA-04 (83.52%) > OPA-09 (63.40%). Out of 5 primers the maximum and minimum polymorphism was observed at 99.9 and 63.40% through OPA-05 and OPA-09 primers (Table 3). The maximum (99.5%) polymorphism out of 5 primers was recorded in *O. niloticus* collected from H3 (Table 3).

3.3. Genetic diversity

The genetic variation in the natural stocks of *O. niloticus* harvested from the Wadi Haneffah showed a decline in the genetic

Table 2
Polymorphic and Monomorphic bands.

Locus	No. of polymorphic bands	No. of monomorphic bands
OPA-02	9	3
OPA-04	15	4
OPA-05	13	0
OPA-08	13	0
OPA-09	13	3
Total	50	10

Table 1
Random decamer primers with their Primer sequence, GC content, and annealing temperature.

Sr. No	Locus	Primer sequence (5′-3′)	G + C (%)	Tm (C)
1	OPA-02	TGCCGAGCTG	60	32 °C
2	OPA-04	AATCGGGCTG	60	32 °C
3	OPA-05	AGGGGTCTTG	60	32 °C
4	OPA-08	AGGGGTCTTG	60	34 °C
5	OPA-09	GTGACGTAGG	60	34 °C

Table 3
Total number of amplified fragments, number of polymorphic bands, and percentage polymorphisms generated by PCR using five primers.

Primers	Band pattern	Populations				Total no. of bands
		WH1	WH2	WH3	WH4	
OPA-02	P	1	4	2	2	9
	M	0	2	1	0	3
	%P	100.00	66.66	70.23	100.00	84.22%
OPA-04	P	5	5	3	2	15
	M	1	2	1	0	4
	%P	83.33	71.42	75.00	100.00	82.43%
OPA-05	P	4	4	3	2	13
	M	0	0	0	0	0
	%P	100.00	100.00*	100.00	100.00	100.00%
OPA-08	P	1	5	4	3	13
	M	1	0	0	0	0
	%P	50.00	100.00	100.00	100.00	87.50%
OPA-09	P	2	4	3	4	13
	M	2	0	0	0	2
	%P	50.00	100.00	100.00	100*	62.50%
Average %P		76.12%	87.62%	88.75%	100%	

P - Polymorphic bands; M - Monomorphic bands.
* Primer showing highest percent polymorphism**Primer showing lowest percent polymorphism.

variation due to water pollution and other human activities, genetic drift, and inbreeding.

3.4. Allelic frequency

"Allelic frequencies for each locus in the population of *O. niloticus* collected from four different locations is given" in Table 4. Null alleles were observed with a value of 1 in the fish harvested from H1, H2, H3 and H4.

3.5. Heterozygosity

The heterozygosity was ranged from 0.380 to 0.475. The heterozygosity was 0.380, 0.468, 0.463, and 0.475 in *O. niloticus* collected from H1, H2, H3, and H4, respectively (Table 5).

3.6. UPGMA cluster analysis

The phylogenetic tree exhibited the presence of 3 clusters (Fig. 1). The first cluster, second and third cluster was formed

Table 4
Allele frequencies in four populations of *Oreochromis niloticus* at five loci.

Locus Name	Populations							
	WH1		WH2		WH3		WH4	
	#Obs	Allele frequency	#Obs	Allele frequency	# Obs	allele frequency	# Obs	Allele frequency
OPA-02	24	0.565	26	0.642	26	0.645	27	0.687
	6	0.435	4	0.358	4	0.355	3	0.313
OPA-04	27	0.701	26	0.650	26	0.646	26	0.640
	3	0.299	4	0.350	4	0.344	4	0.360
OPA-05	27	0.672	25	0.597	26	0.627	27	0.687
	3	0.328	5	0.403	4	0.373	3	0.313
OPA-08	4	0.092	26	0.642	26	0.632	26	0.637
	26	0.908	4	0.358	4	0.368	4	0.363
OPA-09	30	1.0000	30	1.0000	30	1.0000	30	1.0000
	0	0.0000	0	0.0000	0	0.0000	0	0.0000
Null alleles	1		1		1		1	

Table 5
Heterozygosity values in four populations of *Oreochromis niloticus* at five loci.

Locus name	Populations							
	WH1		WH2		WH3		WH4	
	#hets	het freq	# hets	het freq	# hets	het freq	# hets	het freq
OPA-02	15.31	0.495	13.96	0.465	12.95	0.436	13.94	0.467
	15.31	0.495	13.96	0.465	12.95	0.436	13.94	0.467
OPA-04	13.33	0.431	13.97	0.467	13.93	0.466	13.93	0.469
	13.33	0.431	13.97	0.467	13.93	0.466	13.93	0.469
OPA-05	13.30	0.430	13.98	0.469	12.99	0.437	14.54	0.487
	13.30	0.430	13.98	0.469	12.99	0.437	14.54	0.487
OPA-08	4.83	0.158	13.97	0.468	13.94	0.469	13.95	0.470
	4.83	0.158	13.97	0.468	13.94	0.469	13.95	0.470
OPA-09	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000
Average Heterozygosity	0.378		0.467		0.452		0.473	

hets: heterozygosity; het freq: heterozygosis frequency.

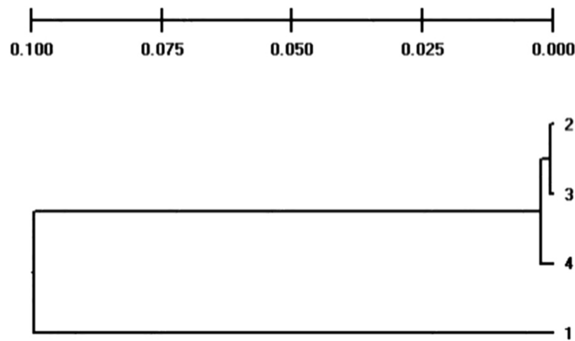

Fig. 1. Dendrogram construction based on Nei's genetic distance among *Oreochromis niloticus* populations.

between the H4 and H3, the H2 and H3 tilapia stocks, and the fish collected from H1 showed a separate cluster. Genetic distance was highest in the fish harvested from H1 and H3, which showed a heterozygous genotype. "The minimum heterozygosity recorded between the fish collected HM and HK, which showed a homogenate genotype. Node distance included population (1) 0.0005, 2, 3 (2) 0.0025 2, 3, 4, and (3) 0.0996, 1, 2, 3, 4."

4. Discussion

O. niloticus harvested from H1, H2, and H3 showed the maximum of genetic variation (99.99) caused by OPA-02, OPA-05, and OPA-08. The fish introgressive hybridization was due to discharge of untreated domestic and industrial waste. Isoenzyme analysis of *O. niloticus* specimens showed genetic diversity in the fish population from the same region and river (Kohlmann and Kersten, 1999). The maximum and minimum polymorphism was recorded as 99.99 and 63.40% by OPA-05 and OPA-09 (Table 2). The polymorphism was higher than the percentage obtained by Li and Chu-Wu (2006) by RAPD analysis. Out of 60 bands, 48 bands showed polymorphism. Basavaraju et al. (2007) reported 57.1% polymorphism in tilapia. Basavaraju et al. (2014) used 8 random primers to study genetic diversity in *L. fimbriatus* and observed polymorphic bands. We had recorded the highest genetic variation in *O. niloticus* collected from H4 location and lowest from H1, which indicates fish from H4 have more heterozygous genotypes. The similar findings were also reported by Chandra et al. (2010).

"Genetic distance ranged between 0.0005 and 0.0996. The highest and lowest genetic was recorded in the fish stocks obtained from H1 and H2, respectively". Ji et al. (2014) studied the genetic distance in five populations of *Megalobrama amblycephala* and reported genetic variation. The highest heterozygosity in *O. niloticus* harvested from H1. A low level of genetic variation was observed in the fish stock collected from four different locations (Gopalakrishnan et al., 2009). These results were not consistent with findings of Kohlmann and Kersten (1999), they mentioned varying diversity in fish stocks. The cluster analysis exhibited the fish collected from H3 was resemble to H4, while the tilapia collected from H1 was genetically distant from the other stocks. Basavaraju et al. (2014) observed two cluster in a group of three different stock. Bartfai et al. (2003) reported no grouping in the stock of common carp. The loss in genetic variation in the fish stocks of *O. niloticus* in Wadi Haneffah, Riyadh, Saudi Arabia due to increased load of domestic and industrial waste. The increasing loss in the genetic variation in the tilapia may decrease its potential to overcome the habitat degradation due to anthropogenic activities (Milligan et al., 1994).

5. Conclusion

It has been concluded that RAPD technique is very useful to collect data about the genetic variation in the wild stock of fish population of the same geographic region. This information may be helpful to plan strategies for improvement in the breeding program by the fisheries biologist.

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