Genetic Polymorphism between Natural Populations of the Nile Tilapia (*Oreochromis niloticus* L.) Based on Randomly Amplified Polymorphie DNA Markers

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ABSTRACT

Nine natural population of Nile Tilapia (*Oreochromis niloticus* L.,) were collected from different governorates in Egypt to calculate the genetic polymorphism with/within the different populations. Collected fish samples were subjected to DNA extraction using arbitrary short (10 nucleotides) primers randomly amplified polymorphie DNA -PCR. In a total of 726 amplification fragments; 430 were polymorphic and 296 were monomorphic with general genetic polymorphism percentage 59.23%. The recorded results indicated that there were high significant variations regarding the population from different location in Egypt. The genetic polymorphism in the different populations were (56.84%) in Marsa Matroh governorate: Siwa oasis (54.43%) and Al-Negelia (59.25%); (60.08%) in Alexandria governorate: Abis (59.34%), Alameryia (60.82%); (59.26%) in El-Beheria governorate: Nobariya (59.21%), Edko (58.02%), Rosetta (60.56%); Kafer Elshikh governorate: Desuq (59.64%) and 59.74% in Aswan governorate. The highest amplification fragments were recorded to OPN-10 (98), OPQ-14 (90) and OPM-5 (86) primers, in respect.

Key words: Genetic polymorphism, populations, Nile tilapia, RAPD.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus* L.,) is the common name for 70 species of perch-like fishes, which belong to the Cichlidae family and are native to the freshwaters of tropical Africa. They include the mouth breeding genera of *Sarotherodon* and *Oreochromis* as well as substrate spawning tilapia (Trewavas, 1983). Nile tilapia has been the fish species with the greatest production expansion in aquaculture in recent years. Worldwide production of Nile tilapia has increased roughly to 1.1 million metric tons, and is thereby only fractionally lower than the production of e.g. salmon was 1.2 million metric tons (FAO, 2001). Some tilapia species are cultured as important fish food in a number of tropical and subtropical countries. At the same time the genetic resources of tilapia have not always been managed well and some species have even become endangered (Agnese *et al*., 1999). Knowledge of the population structure of Nile tilapia is economically important for several issues pertaining the future development of aquaculture strains and management of fishery. Identification of subpopulation (stock) often biologically meaningful geographic boundaries for evaluating a number of criteria, including genetic diversity. The latter is important because subpopulations (stocks) may possess novel genetic, physiological, behavioral and/or other characters that promote noticeable variations in life-history charaters such as growth rates, fecundity, abundance and disease resistance. Various methods of genetic markers for stock identification and assessment in Nile tilapia have historically been used, including: allozymes (Avtalion *et al*., 1976; McAndrew and Majumdar, 1983; Basiao and Taniguchi, 1984; Macaranas *et al*., 1986; Macaranas, *et al.*, 1995; Pouyaud and Agnese, 1995; Rognon, *et al.,* 1996); mitochondrial DNA restriction fragment length polymorphisms (Agnese *et al.,* 1997; Rognon and Guyomard, 1997) and random amplified polymorphic DNA (RAPD) (Bardakci and Skibinski 1994; Naish *et al.,* 1995; Dinesh, *et al.,* 1996; Hassanien *et al.,* 2004).

Genetic markers represent genetic variation between individual organisms or species. Generally, they do not demonstrate the target genes themselves but act as 'signs'or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci'[singular 'locus'] (Hammer *et al*. 2000). Also, DNA markers, reveal sites of variation at the DNA level. These markers have the advantage of being numerous in the nature and not affected by the environment as in the case of morphological markers. The expression of most genes is quantitative in segregating populations and is confounded by the environment (Marshall *et al*. 2001). There are three major types of genetic markers: (1) morphological markers which themselves are phenotypic traits or characters, (2) biochemical markers, which include allelic variants of enzymes called isozymes, and (3) DNA (or molecular) markers, which reveal sites of variation in DNA (Jones *et al.,* 1997)

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MATERIALS AND METHODS

The present experiment was carried out at the Faculty of Agriculture, Saba Basha, Alexandria University, Agriculture Biotechnology laboratory, Alexandria, Egypt, during the year of 2016 to detect genetic polymorphism based on ten RAPD-PCR markers of natural population of Nile tilapia (*Oreochromis niloticus* L.) which collected from different locations in Egypt.

Sample collection for DNA analysis

Nine Nile tilapia (*O. niloticus*) populations were collected from different governorates in Egypt as Marsa Matroh at Siwa oasis and Al-Negelia; Alexandria at Abis and Alameryia; El-Beheria at Nobariya, Edko, Rosetta; Kafer Elshikh at Desuq; and Aswan. Five fish samples were collected naturally and kept in ice box until DNA extraction and analysis.

DNA extraction and polymerase chain reaction (PCR) amplification

RAPD has been developed, in which DNA was amplified by the polymerase chain reaction (PCR) using arbitrary short (10 nucleotides) primers (Williams *et al*., 1990). A set of 10 random10-mer primers selected from the Operon Kit [Operon Technologies Inc., Alabameda, CA] (Table 1) was used in the identification of genetic polymorphism among the different locations from Egypt. Amplification reaction was performed in total volume of 25µl containing 25 ng of genomic DNA, 1X MgCl2 free Taq DNA-polymerase buffer, 2 mM $MgCl_2$, 250 mM each dNTP,10 pmol of a single10-base primer and 0.25U of Taq DNA-polymerase. PCR was performed on a 11000-thermocycler programmed for 94 (1cycle) 5 minutes, then 40 cycles of 40s at 94 (1cycle),1min at 35 (1cycle) and 2 min 72 (1cycle). PCR products were analysed using gel electrophoresis with 2.5% agarose gel and detected by staining with ethidium bromide. RAPD patterns of individuals were compared within and between different populations. Fragments were scored as (1) if present or (0) if absent. Clustering methods and similarity coefficients were tested using the procedures SIMQUAL, SAHN, and TREE from the program NTSYSpc version 2.10 (Applied Biostatistics, Setauket, New York, USA). The clustering methods UPGMA, WPGMA, Complete-link, and Single-link were applied in all possible combinations with the similarity coefficients Dice, Jaccard and simple matching. Rohlf (2000) describes clustering methods and similarity coefficients.

RESULTS AND DISCUSSION

Data in Table (2) demonstrate that the number of amplicons ranged from 55 to 98 fragments for the primer OPD-05 and OPN-10, respectively. In a total of 726; 430 bands were polymorphic and 296 monomorphic bands. Also, genetic polymorphism ranged from 19.04 to 70.00 with OPB-07 and OPQ-14, each inturn. General genetic polymorphism was 59.23% between the tested natural population of Nile tilapia. Data in Table (3) showed clearly the differential between the test tilapia populations. For population from Marsa Matroh governorate which includes Siwa oasis (54.43%) and Al-Negelia (59.25%), the total amplifications fragments ranged from 79 to 81 and the polymorphic bands was 43 to 48 bands with no significant observed variations. Also, the second governorate, Alexandria, expressed almost the same amplification fragments, 91 and 97 for Abis farm (59.34%) and Alameryia city (60.82%). For El-Beheria governorate, Nobariya city recorded 76 band with 59.21%; Edko city (81 bands) with 58.02% and 71 bands for Rosetta city by 60.56% genetic polymorphism. Finally, for Kafer Elshikh Governorate, 73 band detected for Dosoq city by 59.64% and 77 amplifications fragments with genetic polymorphism 59.74% in Aswan governorate. Cluster analysis in Figure (1) divided the nine-natural populations of Nile tilapia in to two main groups with 89% genetic similarity. The first group includes Abis and Alameryia city with 96% while the second group includes two sub group; Siwa and Al-Negelia (98%), in one group, Rashied, Edko and Dosoq (97%) and finally Nobaryia and Aswan in the last one (99%).

In the same contrast Hassenien *et al*. (2004) studied the genetic diversity of the Nile tilapa collected from the river nile and delta lakes in Egypt, by the analysis of randomly amplified polymorphic DNA (RAPD). Their results showed that of 25 primers examined, 21 primers produced 230 RAPD bands and the percentage of polymorphic bands in Manzalla lake was (29.4%) and Burullus was (24%) populations was low compared with Assuit (30.54%), Cairo (33.5%) and Qena (44.84%) populations. The highest percentage of polymorphic bands was observed in the Qena population, suggesting a greater potential for application in breeding programs. RAPD analysis was applied to three species of the tilapia genus *Oreochromis* and four subspecies of *O. niloticus* by Bardakci and Skibinski (1994). Thirteen random10-mer primers were used to assay polymorphisms within and between populations. Different RAPD fragment patterns were observed for different species, although not always for different subspecies. Evidence was presented that RAPD markers may be useful for systematic investigations at the level of species and subspecies. Dinesh *et al.* (1996) used RAPD fingerprinting for estimating genetic variation and species differentiation in three species of tilapia. A total of 13 RAPD markers differentiating the three species of tilapia were detected.

Table 1. Primers name and their oligonucleotide sequences used in the current study

primer number	primer Code	Sequence
1	OPN-04	5`- GACCGACCCA -3`
2	OPD-05	5`- TGAGCGGACA -3`
3	OPC-05	5`-GATGACCGCC -3`
4	OPM-05	5`- GGGAACGTGT -3`
5	OPB-07	5`-GAAACGGGTG -3`
6	OPN-10	5`-ACAACTGGGG -3`
7	OPG-12	5`-CAGCTCACGA -3`
8	OPQ-12	5`- AGTAGGGCAC -3`
9	OPN-13	5`-AGCGTCACTC - 3`
10	OPQ-14	5`-GGACGCTTCA -3

Table 2. Numbers of both amplicons and the number of polymorphic bands produced by each RAPD primer for ten populations of Nile tilapia

Primers	Number of amplicons	Polymorphic amplicons	Monomorphic amplicons	Polymorphism %
OPN-04	64	33	31	51.56
OPD-05	55	37	18	76.27
OPC-05	76	40	36	52.63
OPM-5	86	54	32	46.44
OPB-07	56	34	22	19.04
OPN-10	98	56	42	57.14
OPG-12	77	44	33	57.14
OPQ-12	65	35	30	53.84
OPN-13	59	34	25	57.62
OPQ-14	90	63	27	70.00
Total	726	430	296	59.23

Table 3. Total amplifications, number of polymorphic bands generated per ten primers within nine populations of Nile tilapia

Primers	Populations									Total
	Siwa	Al-Negelia	Abis	Alameryia	Nobariya	Edko	Rosatta	Desuq	Aswan	
	Number of Polymorphic Fragments									
OPN-04	3/7	3/7	6/9	6/9	3/7	3/7	3/7	3/6	3/5	33
OPD-05	2/5	3/5	6/7	6/9	5/7	4/6	3/6	4/5	4/5	37
OPC-05	5/9	5/10	5/9	6/11	4/6	5/9	3/5	4/9	3/8	40
OPM-5	5/11	7/10	7/12	7/11	5/8	8/11	3/5	6/8	6/10	54
OPB-07	3/5	3/5	3/7	5/8	4/6	2/4	6/8	5/7	3/6	34
OPN-10	7/10	7/12	8/15	8/16	6/10	6/10	4/8	4/8	6/9	56
OPG-12	4/7	4/7	3/7	4/8	3/6	7/12	7/12	7/10	5/8	44
OPQ-12	4/8	4/7	6/10	6/10	5/11	4/8	3/5	1/3	2/3	35
OPN-13	2/4	1/4	2/5	3/5	5/7	3/6	5/7	6/10	7/11	34
OPQ-14	8/13	11/14	8/10	8/10	5/8	5/8	6/8	5/7	7/12	63
Total	43/79	48/81	54/91	59/97	45/76	47/81	43/71	45/73	46/77	726
Polym. %	54.43	59.25	59.34	60.82	59.21	58.02	60.56	61.64	59.74	59.22

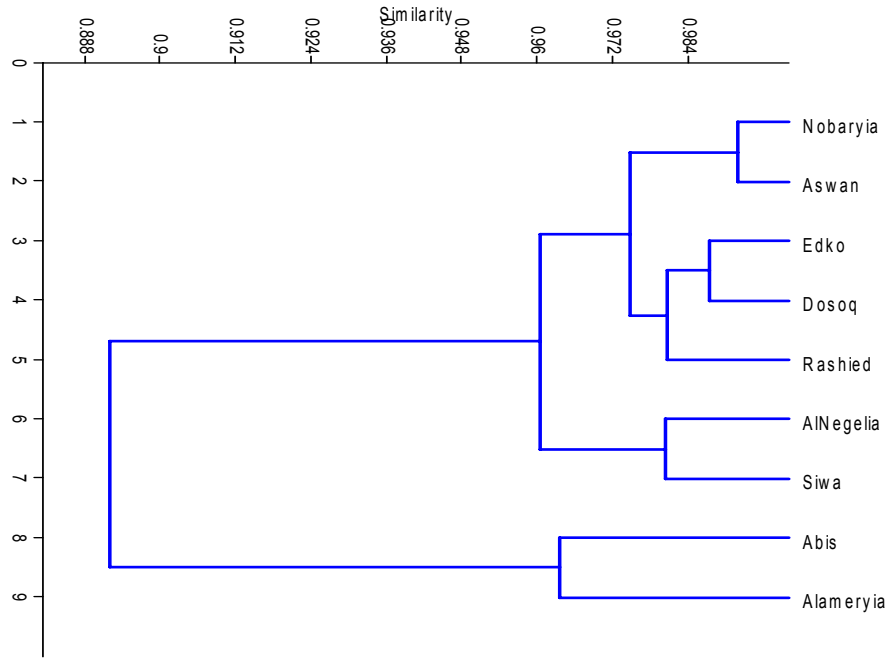

Figure 1. Cluster analysis and genetic similarity of nine natural populations of Nile tilapia in Egypt

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الملخص العربي

تعدد الاشكال المظهرية بين العشائر الطبيعية من سمك الباطى النيلى اعتمادا على المعلمات العشوائية

اسماء احمد خالد

تم تجميع تسعة عشائر طبيعية من اسماك الباطى النيلى من خمس محافظات مختلفة فى مصر بغرض تحديد مـدى التنوع الوراثى وتعدد الشكال المظهرية بين تلـك العـشائر المختلفة. حيث جمعت عينات الاسماك وحفظت فى مبـرد لحين استخلاص المادة الوراثية تمهيـدل لتفاعـل البلمـرة المستمر واستخدان تكنيك القطع العـشوائية باسـتخدام ١٠ معلمات مختلفة. اوضحت النتائج من مجمـوع كلـى ٧٢٦ قطعة جينية ان هناك ٤٣٠ موقع كان بيـنهم تعـدد فـى الاشكال المظهرية و٢٩٦ لا يوجد تعـدد مظهـرى بيـنهم وبنسبة عامة ٥٩,٢٣% تعدد مظهرى. كما اوضحت النتائج ان هناك تنوع وراثى ملحوظ بين العشائر الطبيعية حيـث سجلت محافظة مرسى مطروح ٥٤,٤٣% تلهـا محافظـة الاسكندرية ٦٠,٨% ومحافظة البحيرة ٥٩,٢٦% ومحافظة كفر الشيخ ٥٩,٦٤% واخيرا محافظة اسوان ٥٩,٧٤% كما سجل المعلم الـوراثى OPN-10 ٩٨ قطعـة جينيـة يليـة OPQ-14 سجل ٩٠ قطعة ثـم OPM-5 سـجل ٨٦ قطعـة جينية.