Genetic Differentiation Between Sex Reversal and Normal of Full-Sib Nile Tilapia, *Oreochromis niloticus* Based on DNA Fingerprinting

1Samy Y. El-Zaeem and 2Mohamed M.M. Ahmed

1Department of Animal and Fish Production, Faculty of Agriculture, Saba-Bacha, Alexandria University, Alexandria, Egypt.
2Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City For Scientific Research and Technology Applications, Alexandria, Egypt.

**Abstract:** The identification of sex reversal and normal of full-sib Nile Tilapia (*Oreochromis niloticus*) consider a great potential for detection the commercial deceit and protection of human health. Random amplified polymorphic DNA (RAPD) analysis was applied to identify of sex reversal and normal of full-sib Nile Tilapia. Eleven random primers were used to assay polymorphisms between these fish. The results showed that high polymorphic percentage (55.76%) were detected between sex reversal and normal of full-sib Nile Tilapia using different random primers. The results supported also the hypothesis that the DNA diversity may be happened in the genome of Nile Tilapia fed on 17α-methyltestosterone (17α-MT) as hormone sex reversal.

**Key words:** Nile Tilapia, Genetic differentiation, sex reversal, RAPD, fingerprint.

**INTRODUCTION**

Tilapia are cultured throughout the world because they grow fast, are easy to feed, are resistant to poor water quality and disease and are easily reproduced[1,2]. A major problem in tilapia culture is precocious maturation, which results in overcrowding and stunted growth of the original stock. Males have greater growth potential than females[3] and research has led to the production of all-male fish using the hormone treatment of 17α-methyltestosterone (17α-MT) to alter the sex to males[4-7].

However, this technique suffers from some limitations such as the consumer's concern for the carcinogenicity and sex interference of the administered androgen and its residues[8]. Therefore, commercialization of this technique was subjected to intense investigation in several countries. Recently, in Egypt, the General Authority for Fish Resources Development (GAFRD) prevent the usage of hormone sex reversal for commercial production of fish. However, there are many fish farms have been producing the hormone sex reversal tilapia as food for human. Therefore, the identification of these fish consider a great potential for detection the commercial deceit and protection of human health.

The technique of random amplified polymorphic DNA (RAPD) marker[9,10] has been successfully exploited for stock identification and population analysis in fish[11-15] and mollusks[16,17], analysis of population structure in black tiger shrimp[18], marine algae[19], analysis of genetic impact of environmental stressors[20] and analysis of genetic diversity[21-23]. The main advantages of RAPD markers are the possibility of working with anonymous DNA and the relatively low expense[24], also fast and simple to produce RAPD marker[25]. Therefore, the aim of this work to employ the RAPD marker to study and identification the molecular differences between sex reversal and normal of full-sib Nile Tilapia, *Oreochromis niloticus*.

**MATERIALS AND METHODS**

The present study was carried out at Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University & Nucleic Acid Research Department (GEBRI), Mubarak City for Scientific Research & Technology Applications, Alexandria, Egypt.

**Fish origin:** The Nile Tilapia, *Oreochromis niloticus* broodstock used in this study descended from a randomly mating population at the experimental fish farm, Faculty of Agriculture (Saba-Bacha), Alexandria University, Alexandria, Egypt.

**Fry production:** Nile Tilapia, *O. niloticus* spawners (1 male and 1 female) were maintained in a rectangle fiberglass tank (350l). The tank was supplied with fresh water and continuous airation. Water temperature was thermostatically regulated and fixed at 28°C. Readiness of female to spawn ascertained by examining the degree of swelling of the urogenital papilla[26]. Also, male was examined by the stripped out of the male sperm[27].

Fry were collected after 10 days from the mouth of female and divided randomly into two groups. Each group was maintained separately in a glass aquarium.
(100x34x50 cm). Each aquaria was supplied with fresh water and adequate continuous aeration systems and water temperatures were thermoregulated at 28°C. Also, these aquaria were cleaned by siphoning, then water was partially changed once daily.

**Hormonal treatment:** Sex reversal to males was attempted using the synthetic steroid 17 α-methyltestosterone (17 α-MT). The 17 α-MT was dissolved in 96% ethanol. The solution was mixed with diet (40% protein) at a level of 60 mg/kg of diet as described by[28,30]. Diet was air dried overnight at room temperature. Control diet prepared in exactly the same manner as the hormone diet (with hormone exclusion). In all treatments fry were fed to satiation at least twice per day for 28 days commencing at first feeding[30].

**Sample preparation:** By the end of hormonal treatment, a random sample of fifty *O. niloticus* fingerlings from each group (hormone treated and control) were dissected and their liver were carefully removed and stored at –20 for DNA extraction.

**DNA isolation:** DNA was extracted from fine tissue (liver) following the method described by[31]. Approximately 50 mg of the liver tissue was homogenizer in mortar and suspended in 500 µL STE (0.1 M NaCl, 0.05 MTris and 0.01 M EDTA, pH 8). After adding 30 µL 10% SDS and 30-µl proteinase K (10 mg/mL) the mixture was incubated at 50°C for 30 minutes. DNA was purified by successive extraction with phenol: chloroform: isoamyl alcohol (24:1) respectively. DNA was precipitated with 1.6 volumes of isooamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1) respectively. DNA was precipitated with ice-cold absolute ethanol and washed with 70 % ethanol. The pellet was dried and resuspended in 200µl water.

**PCR Primers:** In this work ten and twenty base long oligonucleotide primers were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification (Table 1).

**PCR amplification and agarose gel electrophoresis:** PCR amplifications were performed following the procedure of[30,32]. The reaction (25 mL) was carried out in a consisted of 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs and 25 pmol of random primer, 2.5 mL. 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (ependorff). The PCR programme included an initial denaturation step at 94°C for 2 mins followed by 45 cycles with 94°C for 30 seconds for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72°C for 10 min were carried out. The samples were cooled at 4°C.

**Table 1:** The sequences, GC% and the annealing temperatures of the primers used.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence 5´-3´</th>
<th>GC%</th>
<th>Annealing °C</th>
<th>Sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AGG CCC CTG T</td>
<td>70</td>
<td>30/30</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ATG CCC CTG T</td>
<td>60</td>
<td>30/30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AAA GCT GCG G</td>
<td>60</td>
<td>30/30</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ACC GCC GAA G</td>
<td>70</td>
<td>30/30</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CAG GCC CTT CCA GCA CCC AC</td>
<td>70</td>
<td>52/30</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>GAA ACG GTT GTT GAT GCG AG</td>
<td>60</td>
<td>58/30</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>GTG GAC GCA GGG GTA AGC CC</td>
<td>70</td>
<td>54/30</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>GGA CTT GAG TGT GAT GCG AG</td>
<td>60</td>
<td>58/30</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>GGA CTT GAG TGG TGA CGC AG</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>GAA TGG GAC G</td>
<td>60</td>
<td>40/30</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>ATG ACG TGG A</td>
<td>40</td>
<td>40/30</td>
<td></td>
</tr>
</tbody>
</table>

**Agarose Gel Electrophoresis:** The amplified DNA fragments were separated on 2.5% agarose gel and stained with ethidium bromide. 100 bp DNA Ladder marker (bp 2000, 1500, ...500, 400, 300, 200, 100) was used in this study. The amplified pattern was visualized on an UV transilluminator and photographed by Gel Documentation system.

**Scoring and analysis of RAPDs:** The DNA bands were scored for their presence (1) or absence (0) in the RAPD profiles. The index of similarity between sex reversal and normal of full-sib Nile Tilapia was calculated using the formula: \(B_{ab} = 2 N_{ab}/(N_a + N_b)\), where \(N_a\) is the number of common fragments observed in individuals a and b and \(N_b\) and \(N_a\) are the total number of fragments scored in a and b respectively, similarity plus polymorphic equal one[33].

**RESULTS AND DISCUSSION**

All the eleven different primers used in this study, produced different RAPD band patterns (Table 1). The number of amplified bands detected varied, depending on the primers and treatments. Moreover, to ensure that the amplified DNA bands originated from genomic DNA, not from primer artifacts. Also, negative control was carried out for each primer / treatment combination. No amplification was detected in the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Table 2 and Fig. 1).

Random amplified polymorphic DNA (RAPD) analysis was used for detection the genetic differentiation between sex reversal and normal of full-sib Nile Tilapia (*O. niloticus*). The results showed highly genetic polymorphic percentage range (0.00 to 100 %) with an average (55.76%) using different random primers (Table 2 and Figure 1). The results supported also the hypothesis that the DNA diversity may be happened in the genome of fish fed on 17 α-methyltestosterone (17 α-MT) as hormone sex reversal. Moreover, the identification of sex reversal Nile Tilapia consider a great potential for detection the commercial deceit and protection of human health. The results showed also that,
Fig. 1: RAPD amplification products generated from sex reversal and normal of full-sib Nile Tilapia using eleven random primers (P1-P11). M: DNA marker 100 bp ladder; Lane 1: sex reversal; Lane 2: normal, respectively.

Table 2: The percentage of polymorphic between sex reversal and normal of full-sib Nile Tilapia using different random primers.

<table>
<thead>
<tr>
<th>Primers No.</th>
<th>Polymorphic bands %</th>
<th>No polymorphic bands</th>
<th>No. total bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>66.67</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>66.67</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Average</td>
<td>55.76</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

the RAPD assay was successfully used to detect the genetic diversity between sex reversal and normal of full-sib Nile Tilapia\(^{[11-15, 34]}\).

Nevertheless, the specific characteristics of the RAPD method (random, uncharacterized multiple genome loci; dominant nature of markers; and possibility of comigrating, nonhomologous bands) result in limitations based on RAPD analysis alone. Despite these limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species\(^{[35]}\). This work represents a first step towards the generation of DNA markers for purposes, such as species diagnosis, detection of molecular markers linked to economic traits, assessment of genetic diversity and studies on molecular systematic. This is the first report on the use of RAPD markers for studying genetic polymorphic between sex reversal and normal of full-sib Nile Tilapia. Further studies with other molecular methodologies are essential to clarify and confirm to effects of 17 α-methyltestosterone (17 α-MT) on genetic polymorphic between sex reversal and normal of full-sib Nile Tilapia.

REFERENCES


