Genetic Variation Investigation of Tilapia Grown under Saudi Arabian Controlled Environment

O.H. Mohamed Shair, R.M. Al-Ssum and A.H. Bahkali
Department of Botany and Microbiology, King Saud University College of Science, Riyadh, Saudi Arabia

Corresponding Author: O.H. Mohamed Shair, Department of Botany and Microbiology, King Saud University College of Science, Riyadh, Saudi Arabia

ABSTRACT
Introductions of better performing tilapia species and development of techniques to manage unwanted reproduction have made significant developments that led to success in tilapia farming. Samples were obtained from three pools at Dirab fish station, in Saudi Arabia. The genomic DNA of fish was extracted from 4 μL of whole blood following the method described by pharmacia Biotech. DNA amplifications were performed by using PCR program consisting: denaturation temperature 94°C for 4 min and 40 cycles consisting: 94°C denaturation for 30 sec, annealing temperature 36°C for 1 min, extending temperature 72°C for 2 min. Out of 12 primers one was selected for PCR reaction using genomic fish as template. The total band profiles of sample fishes from first three pools and that of the second three pools were counted. The similarity index between populations, Oreochromis aureus and Oreochromis mossambicus and Oreochromis niloticus were calculated by using the formula, SI = 3NABC /(NA+NB+NC) where NABC was the total number of RAPD bands shared by individuals A and B, C and NA, NB and NC were the number of bands scored. The mean band profiles of all individuals were also calculated as 8.1 and 8 and the Similarity Index (SI) values between the band profiles of all individuals on the same gel were 1.479 and 1.5.

Key words: Dirab, food, source, genetic, diversity

INTRODUCTION
Dirab, is very close to Riyadh, the capital of Saudi Arabia. We were informed by the occupants at this station that in each of the pools sampled were growing Oreochromis aureus, Oreochromis mossambicus and Oreochromis niloticus which were imported from Pertain 20 year ago approximately. Tilapias have a mild, white flesh that sustains them. They don't travel like salmon and these appeals to consumers, making them economically important as a fish food source.

Several commercially important fish stocks exhibit significant year by year biomass fluctuation though the variations are not necessarily reflected variations in individual growth parameters (Eide and Wikan, 2010).

Random Amplified Polymorphic DNA (RAPD) was used to profile the genetic diversity of population (Fouz et al., 2007). The RAPD offered a quick and inexpensive molecular tool that assisted to distinguish, monitor and manage the genetic diversity of natural populations of fish raised in fish hatcheries fish (Brahmane et al., 2006). It is fundamentally important for species conservation as there is a need to adopt new fisheries production strategies to improve gross production as well as efficiency of the production units. This will involve devising new production
methods as well as the adoption of modern effective technologies in aquaculture (Wenne et al., 2007). Amplified fragment length polymorphism (AFLP) is a rapid PCR-based DNA fingerprinting technique that has resolved the phylogeny of recently evolved species complexes in freshwater fish (Li and Chu-Wu, 2007). The use of Simple Sequence Repeats (SSR) methods has attracted researchers' attention for their rich polymorphism stability and reliability (Wu et al., 2009). Future fisheries and fresh water living resources-related research should be directed towards four broad areas of national importance: (1) Develop a scientific basis for managing freshwater resources; (2) understanding the freshwater environment, (3) leveraging scientific understanding to bolster food production efforts and (4) understanding the nature of freshwater biodiversity and its application for human welfare (Gonzalo et al., 2005). Due to the economically importance of tilapia, strong focus on its production as a food source has targeted aquaculture sectors that contribute to the achievement of high quality production values and increased bulk production (Gregory et al., 2007).

*Oreochromis niloticus* is remarkable for its functional versatility and labile ecology, together with its great adaptability to virtually all tropic and subtropics environments, explains its position among the top aquaculture species in the world (Mahrous et al., 2006). DNA-fingerprint similarity is generally defined as the fraction of shared bands for individuals. It is the number of common fragments in their fingerprint Profiles divided by the average number of fragments exhibited by both individuals (Lynch, 1990). Accordingly to Lynch's definition of DNA fingerprint, the similarity index of fish populations in this study was calculated. The species used in this study were from aquaria at Dirab, 45 km from Riyadh, Saudi Arabia. To insight the genetic structure of this tilapia population at Dirab farm, to effectively manage and conserve their structure is essential and is the scope of this study.

**MATERIALS AND METHODS**

Species, *Oreochromis aureus* *Oreochromis mossambicus* and *Oreochromis niloticus*, were the materials used to extract DNA. The tilapias whole blood samples were obtained from Dirab fish station during June 2009 and the project was conducted at King Saud University, college of Science Department of Botany and Microbiology. Following the method described by pharmacia Biotech (Genomic prep blood DNA Isolation Kit). Four microliter of whole blood were added to 600 μL of cell lysis solution containing in a 1.5 mL tube. Quickly were pipette up and down 3-5 times to lyse the cells and were incubated at 37°C until the solution was homogeneous. Three microliter of Rnase A solution were added to the cell lysate. The sample were mixed by inverting the tubes 20 times and incubated at 37°C for 15 min and were cooled to room temperature, added 200 μL of protein precipitation solution, vortexed at high speed vigorously and Precipitated at 13,000 g for 3 min. The precipitated protein formed a tight dark brown pellet. Carefully, the supernatant containing the Genome were poured into a clean 1.5 mL tube containing 600 μL of 100% isopropanol and mixed the sample by inverting gently 60 times until the white threads of the genome formed a visible clump and centrifuged at 13,000 g for 1 min. The supernatants were poured off, drained on clean absorbent paper. The genome was washed with 70% ethanol. The tubes were drained on clean absorbent paper and allowed the sample to air dry for 15 min. The DNA was hydrated by adding 200 μL of genome hydration solution over night at room temperature and stored at 4°C. DNA amplification were performed by using DNA Thermal cycle 480 with a PCR program consisting: denaturation temperature 94°C for 4 min and 40 cycles consisting: 94°C denaturation for 30 sec, annealing temperature 36°C for 1 min, Extending temperature 72°C for 2 min. The PCR products
of each primer were separated by electrophoresis on 1.5% agarose gel. After staining the gel with ethedium bromide, the PCR products were visualized on U.V-transilluminator and documented. RAPD primer2 ('5 AAGAGCCCGT 3') used was purchased from Amersham pharmacia Biotech.

RESULTS

Figure 1 shows three replicates of fish samples from pool one. From left to right of each three Lanes contained Oreochromis aureum Oreochromis mussambicus and Oreochromis niloticus respectively. Figure 2 indicates also three replicates of fish samples from pool two. From left to right of each three lanes of Fig. 2 are contained Oreochromis aureum Oreochromis mussambicus and Oreochromis niloticus, respectively. The results of band profiles scored by each fish in the population of pool one and pool two from top to bottom were 73 and 72, respectively and has been described in Table 1. Figure 3 visualised representation of a relationship between variables of number of bands, the vertical Y axes and population of fish Oreochromis aureus and Oreochromis mussambicus and Oreochromis niloticus horizontal X axes.

Fig. 1: From left ro right 1 to 2 each three lanes contain samples of O. aureus, O. musambicusn and O. nilotcus

Fig. 2: From left ro right 1 to 9 each three lanes contain samples of O. aureus, O. musambicusn and O. nilotcus
Table 1: From left to right top to bottom are band profiles of O. aureus, O. mussambicus and O. niloticus

<table>
<thead>
<tr>
<th>O. aureus</th>
<th>O. mussambicus</th>
<th>O. niloticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Bottom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

![Band profiles of fishes from pool one and two](image)

Fig. 3: Band profiles of fishes from pool one and two

Mean band profiles of Oreochromis aureus Oreochromis mussambicus and Oreochromis niloticus populations were 8.1 and 8. The standard deviation of fish population in the pools ranged 1.8 and 2.2. The similarity index was calculated by using the formula, \( SI = 3NABC / (N_A+N_B+N_C) \) where NABC was the total number of RAPD bands shared by individuals A and B, C and N_A, N_B and N_C were the number of bands scored for each individual, respectively (Lynch, 1990). The Similarity Index (SI) values between the RAPD profiles of all the individuals on the same gel were 1.479 and 1.5.

DISCUSSION

Tilapias were an important component of subsistence fisheries for thousand of years but have gained prominence in recent years in areas where they are not endemic. Fisheries, particularly those small scale sectors characterized by the use of low technology gear over limited range, are fundamentally important in many developing region. They provide important source of protein. The world has to make remarkable progress in increasing fish production towards reducing food insecurity and generate economic benefits including employment for people (Sotul, 2011).

Introductions of better performing technique such as microsatellite and mitochondrial DNA analysis of tilapia species/strains and development to manage unwanted reproduction have spurred significant developments that led to success in tilapia farming (Assem et al., 2005).

Restriction Fragment Length Polymorphism (RFLP) technique requires more DNA relatively pure, specific DNA probes and to detect one must use radioactive isotopes. Randomly Amplified Polymorphism DNA (RAPD) technique permits detection of DNA polymorphism by randomly amplified multiple regions of the genome by polymerase chain reaction using single arbitrary primers designed independently of the target DNA sequence (Salem et al., 2005). Study curried to
investigate the genetic variation in *Prochilodus margravii* by using RAPD revealed that fishes in a downstream region nearest a dam had a higher similarity coefficient (Hatanaka and Galetti, 2003). DNA fingerprinting analysis based on microsatellites was applied by Fopp-Bayat (2009) for separation of mixed gynogenetic offspring of Siberian sturgeon (*Acipenser baeri*) and individuals from commercial production.

This study investigates three species of tilapias: *Oreochromis aureus*, *Oreochromis niloticus* and *Oreochromis mossambicus* adopted in controlled environment in Saudi Arabia by using Randomly Amplified Polymorphism DNA (RAPD) technique. The short ten nucleotide primers generated discrete DNA amplified fragments of varying lengths and revealed RAPD variation among the species. The RAPD bands profile for different individuals of different species as seen in the Fig. 1 and 2 indicated specificity of the DNA patterns for a given species. We have found that the primers of same length but with different sequences generated different DNA patterns among fishes. This technique could be manipulated by changing the primer sequence and length to generate amplification products of desired complexity to suit different purposes like genetic mapping or genotyping depending on the particular primer and template combination. The fingerprints generated by the primers used in this study revealed unique profiles for each species in terms of number and position of RAPD bands. The results obtained in this study showed that RAPD could be used to generate useful fingerprints characteristic of fish species and for genotyping of individuals within the species. Thus, it provides an efficient and sensitive method which can be used to estimate genetic variability, relatedness, inbreeding levels, pedigree analyses, detection of economic traits and in other marker based studies in fishes. According to our statistical analysis of the data obtained, we conclude that, the standard deviation values of pool one and two population ranged between 0.8 and 2.08, respectively within an average mean of 8.1 for pool one and 7.96 for pool two. All of these values are slightly significant statistically indicating low level of genetic divergence in pool two, although the similarity index between the pools calculated, 1.479 for pool one and 1.5 for pool two indicated the non significance of genetic divergence between the populations in study. Nevertheless RAPD in this study provided baseline information on the population structure of *O. aureus*, *O. mossambicus* and *O. niloticus* from Dirab station. We suggest for further studies using mitochondrial and microsatellite to further enhance our understanding of the genetic stock structure of Dirab station fish population. The results of DNA fingerprinting of this study may be taken into consideration as a joint patterns of segregation and probability of hybridization between the much closed species to improve the genetic characters.

ACKNOWLEDGMENTS

My thanks are extended to the members of the research Center; College of Science King Saud University for their financial support. I cannot forget Dr. Rasheed Musa Al-ssum who supervised this work and provided continuous and most valuable criticism and suggestions. I would like to express my sincere gratitude and deep gratefulness to Prof. Ali Bakhali, chairman of the Department of Botany and Microbiology College of Science, King Saud University for his encouragement. My thanks are due to all of the staff members in the Department of Botany and Microbiology for their support and carefulness. Warm and endless thanks to all my colleagues who supported me.

REFERENCES


