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Growth comparison of Nile tilapia (*Oreochromis niloticus*) and Blue tilapia, (*Oreochromis aureus*) as affected by classical and modern breeding methods

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This study was conducted to compare and evaluate the productive performance characteristics of the base generation (F_0) of Nile tilapia, *Oreochromis niloticus* and Blue tilapia, *Oreochromis aureus* under the effect of interspecific hybridization and genetically modified breeding by introducing a fragmented purified DNA isolated from *O. aureus* or *O. niloticus* into the gonads of *O. niloticus* or *O. aureus* parent, respectively. The results showed that the growth performance, body composition and feed utilization parameters of genetically modified *O. aureus* or *O. niloticus* treated with *O. niloticus* or *O. aureus* DNA, respectively were improved significantly ($P \leq 0.05$) as compared to both purebred and interspecific hybridization ($\text{♀ } O. aureus \times \text{♂ } O. niloticus$ and $\text{♀ } O. niloticus \times \text{♂ } O. aureus$). RAPD analysis was used for constructing parsimony tree depicting relationships among the different genotypes studied. The hierarchical cluster analysis based on RAPD fingerprinting, grouped the six genotypes of fish into two major category groups. Within these major grouping, purebred of *O. niloticus*, *O. aureus* and their reciprocal hybrid grouped close together. Also, the dendrogram showed that the hybrid of $\text{♀ } O. aureus \times \text{♂ } O. niloticus$ appear to be more genetically similar to that of the hybrid $\text{♀ } O. niloticus \times \text{♂ } O. aureus$ than that of the purebred of either *O. niloticus* or *O. aureus*. The other major group showed that *O. aureus* injected with *O. niloticus* DNA appear to be more genetic dissimilarity to that of *O. niloticus* injected with *O. aureus* DNA. The results of this study suggested that genetically modified *O. niloticus* and *O. aureus* with higher growth rate can be produced using a feasible and fast methodology compared as to interspecific hybridization.

Key words: Productive performance, *Oreochromis niloticus*, *Oreochromis aureus*, inter-specific hybridization, genetically modified.

INTRODUCTION

Tilapias are of very importance in world fisheries, and are the second most important group of food fishes in the world, next to the carps. Nile tilapia, *Oreochromis niloticus* accounted for a harvest of nearly 2.54 million tones in 2009 (FAO, 2011), second only to carp as a warm water food fish and exceeding the harvest of Atlantic salmon, *Salmo salar*, although, the value of the Atlantic salmon catch is more than twice that of the tilapia catch (Maclean et al., 2002). Although, native to Africa, tilapias are cultured in Asia and the Far East, and occupy two rather separate market niches, being a poor man's food fish in countries such as Israel and the southern United States (Maclean et al., 2002).

Tilapia are easy to culture and reproduce, with rapid sexual maturation at 6/7 months from hatch and marketable at this age. Nile tilapia is also an excellent laboratory animal and deserves to be studied (De la Fuente et al., 1999; Maclean et al., 2002).

Most of the genetically improved strains reaching the aquaculture industry were developed through traditional selective breeding (selection, crossbreeding and hybridization). More emerging modern technologies for genetic manipulation seem to take 10 to 20 years to be established experimentally until applications affect the industry. Thus, chromosome-set and sex manipulations started to affect the industry during 1980's and 1990's.

DNA marker technology and gene manipulations have yet hardly affected the industry. The former have not matured yet, but hold much promise. The latter could have affected the industry already if it was not restricted by public concern (Hulata, 2001). Genetically modified organisms now offer the opportunity to improve both the production and characteristics of conventional strains of animals and plants currently exploited in agriculture and aquaculture. They offer the possibility of a biotechnology revolution representing a further enhancement of agricultural productivity now that the benefits of the so-called green revolution have been assimilated (Kareiva and Stark, 1994; Maclean and Laight, 2000).

Changes in the genetic structure of a population may occur through artificial selective breeding, genetic drift and gene mutation. Mutations occur in natural population at low frequency. However, artificial selection may alter the genetic structure of a population more rapidly. The artificial introduction of a fusion gene to produce a transgenic fish, in theory, is not different from the natural processes, but it is a more rapid approach to transfer new genetic material into a fish (Devlin and Donaldson, 1992).

The American Fisheries Society believes that genetically modified fish can be considered as a special case when it comes to introduction of valuable species. Introduction of fish species relates to artificial movement of wild-type species to new sites for reproduction. Actually, unlike hybrid strain that has a lot of chromosomes, genetically modified fish has almost the same chromosome except a transferred DNA fragment is inserted to generate a specific characteristic. As a result of the large-sized chromosome of fish, there is still a huge possibility for us to select a genetically modified strain in which the foreign gene is integrated into a no features region without jeopardizing other existing genes. As compared to the conventional improvement of fish species by crossing, gene transfer is a much easier, yet effective system. When applied on aquaculture, genetic engineering and gene transfer can be cutting-edge technologies and significant boosts to the fishery industry (Tsai, 2003).

It can be stated that the major differences between traditional and modern breeding methods of fish are three fold; (1) With genetically modified, it has become possible to transfer a single or several genes into fish, while in traditional breeding large parts of the fish genome are changed; (2) traditional breeding is limited to breeding within two different species. Genetically modified breeding is not limited to species barriers. This is possible because DNA is the universal carrier of genetic information in all organisms; (3) genetically modified breeding provide the investigators with the shorten breeding period, possible easy and rapid way for improving fish characteristics (Wang et al., 2001; Dunham et al., 2001; El-Zaeem, 2001, 2004 a, b; El-Zaeem and Assem, 2004; Assem and El-Zaeem, 2005; El-Maremie, 2007; Abd El-Hamid, 2009; Elwan, 2009; El-

Zaeem et al., 2011).

Therefore, this study compared the productive performance of Nile tilapia, *O. niloticus* and Blue tilapia, *Oreochromis aureus* under the effect each of interspecific hybridization and genetically modified breeding through transfer of DNA isolated from *O. aureus* and *O. niloticus* into gonads of *O. niloticus* and *O. aureus*, respectively.

MATERIALS AND METHODS

The experimental work was carried out in the Laboratory of Breeding and Production of Fish, Animal and Fish Production Department, Faculty of Agriculture (Saba-Basha), Alexandria University and Nucleic Acids Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technological Applications, (GEBRI) Alexandria, Egypt.

Fish origin

The Nile tilapia, *O. niloticus* and Blue tilapia, *O. aureus* used in this study descended from a randomly mating population at the Middle East Fish farm, Tolombat Halk El-Gamal, El-Behera Governorate, Egypt. Ripe females and males with an average live weight of *O. niloticus* (70.00 ± 3.63 and 79.15 ± 2.99 g) and *O. aureus* (64.80 ± 2.59 and 74.98 ± 5.70 g), respectively were chosen. Readiness of females to spawn was ascertained by examining the degree of swelling of the urogenital papilla (Hussain et al., 1991). Also, males were examined by the strip out of the male sperm (Wester and Foote, 1972).

Experimental design

Preparation of genomic DNA

High molecular weight DNA was isolated according to Bardakci and Skibinski (1994) method by reducing liver sample from Nile tilapia, *O. niloticus* and Blue tilapia, *O. aureus*. The extracted DNA was restricted by Eco R1 restriction enzyme type II. It was digested with DNA between guanine and adenine according to Tsai et al. (1993). Then, the concentrations of $10 \mu\text{g}/0.1 \text{ ml/fish}$ were adjusted by extrapolating the dilutions for each type of DNA extracted using $0.1 \times \text{SSC}$ buffer.

Injection of genomic DNA into fish gonads

Two males and six females from each Nile tilapia, *O. niloticus* and Blue tilapia, *O. aureus* were injected directly with the foreign DNA. DNA isolated from *O. aureus* was injected into *O. niloticus* and DNA isolated from *O. niloticus* was injected into *O. aureus* gonads using a hypodermic needle. To inoculate the adult fish, the needle was inserted into the openings of oviduct and spermiduct (El-Zaeem, 2001; Lu et al., 2002).

Culture conditions

Purebred of *O. niloticus* and *O. aureus* and their dialed crosses and injected fish with DNA were stocked separately for natural spawning in concrete ponds ($3 \times 1 \times 1.2 \text{ m}$) at a rate of 4 breeders/m^3 . The sex ratio of the fish was 3 females: 1 male. Brood fish were fed twice daily on pellet diet containing 26% protein at satiation for 6

Table 1. The sequences and the annealing temperatures of the primers used.

Primer	Sequence 5` - 3`	Annealing temperature (°C/s)
1	AAA GCT GCG G	28/30
2	ATG CCC CTG T	28/30
3	ACC GCC GAA G	28/30
4	AGG CCC CTG T	28/30
5	AGG GGT CTT G	28/30
6	CCA GCC GAA C	28/30

days a week. Base generation (F_0) offspring of purebred, interspecific hybridization and DNA treatments were produced 2 to 3 weeks after being stocked to spawn. Post-hatching fry produced from each purebred, hybrid and each treatment of DNA were collected and weighed. Then, fry were transferred separately to glass aquaria (With dimensions $100 \times 34 \times 50$ cm) at a rate of 1 fish /10 L. The glass aquaria were provided with a continuous supply of de-chlorinated water and adequate aeration system, cleaned once daily by siphoning, then one-half to two-third of their water volume was replaced. All water was completely changed once every two weeks during fish sampling. Fry were fed three times daily on pelleted diet containing 38% protein to satiation for 75 days. Then, the fish fed to satiation on pelleted diet contained 32% protein till the end of the experiment. Fish were weighed bi-weekly for 120 days.

Quantitative traits measurements

The following parameters were measured: initial and final body weight (g), weight gain (g), specific growth rate (SGR %/day), feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), protein and energy retention percent (PR% and ER%). Gross energy contents of feed were calculated from MacDonald's tables (MacDonald et al., 1973). Gross energy of fish was calculated from their chemical composition using the factor of 5.7 and 9.5 for protein and fat, respectively according to Viola et al. (1981). Initial and final body composition analyses were performed for moisture, crude protein and lipid contents according to the standard AOAC (1984) methods.

Random amplified polymorphic DNA (RAPD) analysis

DNA was extracted from liver tissue of base generation (F_0) of purebred, their dialed crosses and injected fish with DNA following the method described by Bardakci and Skibinski (1994). In this study, ten base long oligonucleotide primers (Table 1) were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification. The polymerase chain reaction amplifications were performed following the procedure of Williams et al. (1990, 1993). The reaction (25 μ l) was carried out in a 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs and 25 pmol of random primer, 2.5 μ l 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Eppendorff). The PCR programme included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 s and final extension at 72°C for 10 min were carried out. The samples were cooled at 4°C. The amplified DNA fragments were separated on 1.5% agarose gel and

stained with ethidium bromide. 100 bp DNA Ladder marker (2642, 1500,...,500, 400, 300, 200, 100 bp) was used in this study.

Moreover, to ensure that the amplified DNA bands originated from genomic DNA, not from primer artifacts, negative control (without DNA source) was carried out for each primer/ treatment combination. The amplified pattern was visualized on an UV transilluminator and photographed by Gel Documentation system.

Scoring and analysis of RAPDs

RAPD patterns were analyzed and scored from photographs. For the analysis and comparison of the patterns, a set of distinct, well-separated bands were selected. The genotypes were determined by recording the presence (1) or absence (0) in the RAPD profiles. Genotype differentiations among the different genotypes of fish based on RAPD fingerprinting were analyzed by means of hierarchical cluster analysis of the SPSS 12.0 (1999) software package. The dendrogram was constructed using the average linkage between groups and the data matrix generated was used for calculation of similarity matrix for all primers based on Jaccard's coefficients method (Jaccard, 1908).

Statistical analysis

Data were analyzed using the following model (Costat, 1986):

$$Y_{ij} = \mu + T_i + B_j + E_{ij}$$

Where, Y_{ij} is the observation of the ij^{th} parameter measured; μ is the overall mean; T_i is the effect of i^{th} dose; B_j is the effect of j^{th} block; E_{ij} is the random error. Significant differences ($P \leq 0.05$) among means were tested by Duncan's multiple range test (Duncan, 1955).

RESULTS

Data of Table 2 showed that initial body weight (IBW) of genetically modified Nile tilapia that received Blue tilapia DNA was significantly ($P \leq 0.05$) increased than those of the other genotypes of fish. Moreover, the highest records of final body weight (FBW) and weight gain (WG) were achieved by genetically modified Nile tilapia that received Blue tilapia DNA, when compared with the other genotypes of fish, but did not differ significantly ($P \leq 0.05$) from that of genetically modified Blue tilapia that received Nile tilapia DNA. While, specific growth rate (SGR %/day) was significantly increased ($P \leq 0.05$) by purebred

Table 2. Growth performance of purebred, interspecific hybridization and genetically modified *O. niloticus* and *O. aureus**.

Genotype	Initial body weight	Final body weight	Weight gain	SGR%/day
<i>O. niloticus</i> (N)	0.27±0.03 ^d	61.29±2.73 ^c	61.02±2.72 ^c	1.96±0.03 ^b
<i>O. aureus</i> (A)	0.19±0.02 ^e	46.89±1.55 ^d	46.71±1.53 ^d	2.00±0.02 ^a
♀ A x ♂ N	0.36±0.03 ^c	70.68±2.24 ^b	70.32±2.21 ^b	1.91±0.02 ^c
♀ N x ♂ A	0.40±0.01 ^c	74.27±3.75 ^b	73.87±3.75 ^b	1.89±0.03 ^c
(A) injected with (N) DNA	0.89±0.02 ^b	96.71±4.82 ^a	95.83±4.80 ^a	1.70±0.01 ^d
(N) injected with (A) DNA	0.97±0.08 ^a	100.26±6.94 ^a	99.29±6.91 ^a	1.68±0.03 ^d

Means having different superscripts within column are significantly different ($P\leq 0.05$). *Survival rates were 100% for all purebred, interspecific hybridization and genetically modified fish; Initial and final body weight (IBW and FBW) = body weight at beginning and end of experiment; Weight gain (WG) = final weight - initial weight; Specific growth rate (SGR% / day) = $(\text{Log}_e \text{final weight} - \text{Log}_e \text{initial weight}) 100 / \text{number of days}$.

Table 3. Body composition of purebred, interspecific hybridization and genetically modified *O. niloticus* and *O. aureus*.

Genotype	Dry matter (%)					
	Moisture %		Crude protein (%)		Crude fat (%)	
	Beginning	End	Beginning	End	Beginning	End
<i>O. niloticus</i> (N)	80.78±0.04 ^a	74.47±0.12 ^a	54.14±0.80	57.02±0.08 ^b	18.07±1.01	24.42±0.31 ^b
<i>O. aureus</i> (A)	80.19±0.57 ^{ab}	74.39±0.14 ^{ab}	53.88±0.19	56.69±0.28 ^b	18.25±0.05	24.14±0.32 ^b
♀ A x ♂ N	79.57±0.15 ^b	73.99±0.04 ^c	53.97±0.13	57.01±0.10 ^b	18.57±0.62	24.38±0.12 ^b
♀ N x ♂ A	79.90±0.20 ^b	74.10±0.04 ^{bc}	53.98±0.30	56.94±0.14 ^b	18.42±0.12	24.37±0.21 ^b
(A) injected with (N) DNA	79.98±0.67 ^b	72.20±0.27 ^d	54.37±0.42	57.39±0.18 ^a	18.29±0.45	25.37±0.20 ^a
(N) injected with (A) DNA	80.12±0.65 ^{ab}	72.37±0.21 ^d	53.83±0.11	57.55±0.18 ^a	18.33±0.15	25.56±0.16 ^a

Means having different superscripts within column are significantly different ($P\leq 0.05$).

Table 4. Feed utilization of purebred, interspecific hybridization and genetically modified *O. niloticus* and *O. aureus*.

Genotype	Feed intake (g)	FCR	PER	PR (%)	ER (%)
<i>O. niloticus</i> (N)	114.67±5.51 ^c	1.88±0.08 ^b	1.65±0.07 ^{bc}	24.05±0.91 ^{bc}	18.49±0.73 ^b
<i>O. aureus</i> (A)	95.00±3.00 ^d	2.04±0.08 ^a	1.54±0.06 ^c	22.42±0.82 ^c	16.98±0.64 ^c
♀ A x ♂ N	128.33±3.51 ^b	1.83±0.01 ^b	1.63±0.02 ^{bc}	24.26±0.29 ^b	19.35±0.07 ^b
♀ N x ♂ A	137.00±6.08 ^b	1.85±0.05 ^b	1.67±0.04 ^b	24.68±0.57 ^b	18.96±0.43 ^b
(A) injected with (N) DNA	158.00±2.65 ^a	1.64±0.06 ^c	1.88±0.08 ^a	30.09±1.27 ^a	23.43±1.00 ^a
(N) injected with (A) DNA	161.00±8.91 ^a	1.62±0.06 ^c	1.91±0.07 ^a	30.51±0.97 ^a	23.79±0.80 ^a

Means having different superscripts within column are significantly different ($P\leq 0.05$). Feed conversion ratio (FCR) = dry feed intake/gain; Protein efficiency ratio (PER) = gain/protein intake; Protein retention percent (PR%) = protein increment (100) / protein intake ; Energy retention percent (ER%) = energy increment (100) / energy intake.

Blue tilapia, there was higher mean when compared with the other genotypes of fish.

The highest record of moisture content at the beginning of the experiment was obtained by purebred of Nile tilapia, showing higher mean, but did not differ significantly ($P\leq 0.05$) from those of purebred Blue tilapia and genetically modified Nile tilapia that received Blue tilapia DNA. While, no significant differences ($P\leq 0.05$) were detected in protein and fat contents among all genotypes of fish at the start of the experiment (Table 3). By the end of the experiment, moisture content of purebred of Nile tilapia showed higher mean when compared with the other genotypes of fish, but did not

differ significantly ($P\leq 0.05$) from that of purebred Blue tilapia. On the other hand, the highest protein and fat contents were significantly increased ($P\leq 0.05$) by genetically modified Nile tilapia that received Blue tilapia DNA, showing higher mean when compared with the other genotypes of fish, but did not differ significantly ($P\leq 0.05$) from that of genetically modified Blue tilapia that received Nile tilapia DNA (Table 3).

Data in Table 4 showed also that feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention percent (PR %) and energy retention percent (ER%) of genetically modified each Nile tilapia and Blue tilapia that received foreign DNA had surpassed

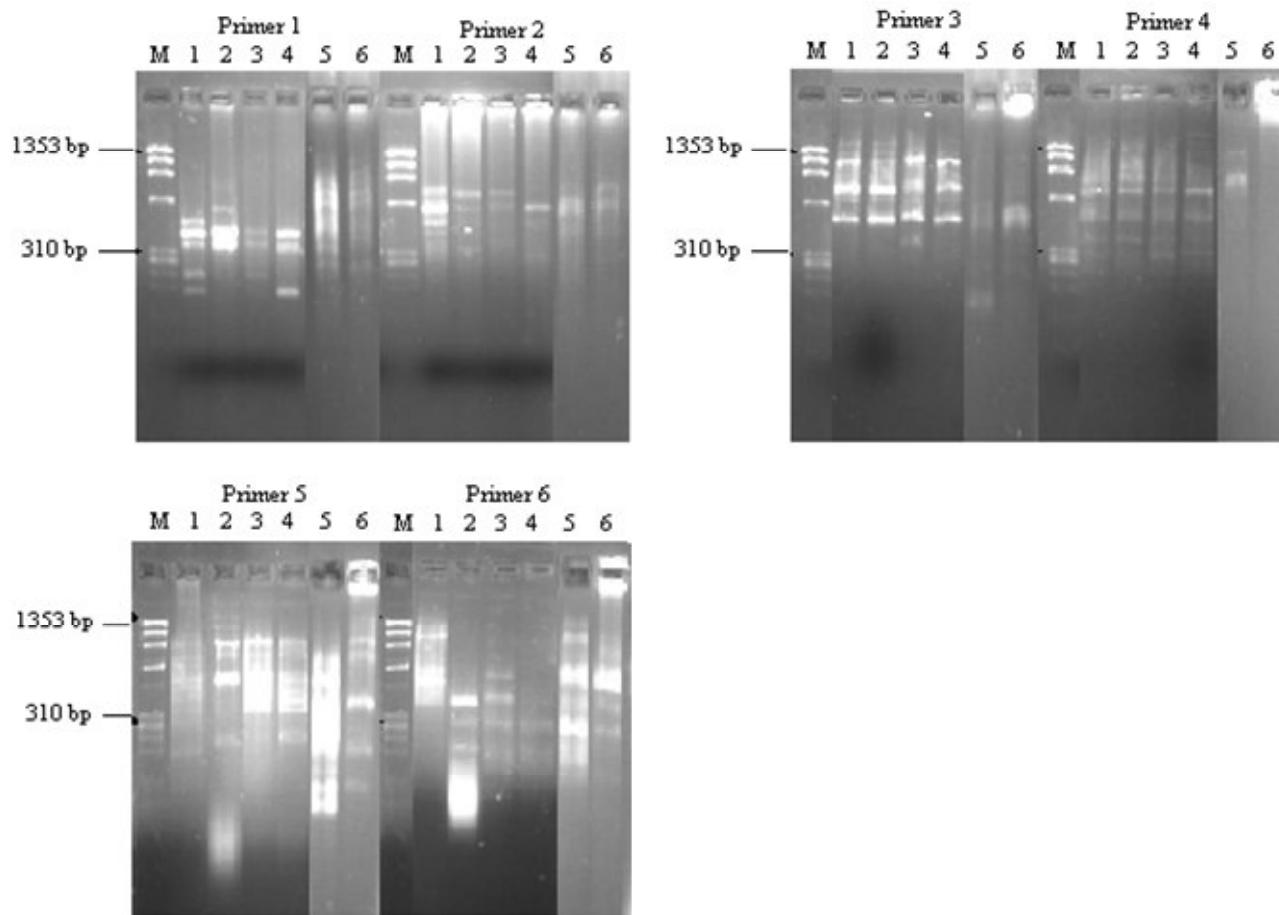


Figure 1. Patterns in different Nile tilapia populations obtained with different primers. Lane M: Φ X174 DNA markers, the lanes (1 to 6) of each primer are: *O. niloticus* (N), *O. aureus* (A), ♀ A x ♂ N, ♀ N x ♂ A, (A) injected with (N) DNA, (N) injected with (A) DNA, respectively.

significantly ($P \leq 0.05$) the purebred and interspecific hybridization.

Considering the results of genotype analysis, all DNA samples from purebred, interspecific hybridization and genetically modified fish were examined using random amplified polymorphic DNA (RAPD) fingerprinting. Six random primers were used to determine DNA fingerprinting diversity in the different genotypes of fish. All the different primers used in this study produced different RAPD band patterns (Figure 1). The number of amplified bands detected varied, depending on the primers and genotypes. The results show that no amplification was detected in the control reactions (without DNA source). All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Figure 1). Moreover, RAPD analysis was used for constructing parsimony tree, depicting relationships among the different genotypes studied. The hierarchical cluster analysis based on RAPD fingerprinting, grouped the six genotypes of fish into two major category groups. Within these major grouping, purebred of *O. niloticus*, *O. aureus* and their reciprocal

hybrid grouped closely together. Also, the dendrogram (Figure 2) showed that the hybrid of ♀ *O. aureus* x ♂ *O. niloticus* appear to be more genetically similar to that of the hybrid ♀ *O. niloticus* x ♂ *O. aureus* than that of the purebred of either *O. niloticus* or *O. aureus*. The other major group showed that *O. aureus* injected with *O. niloticus* DNA appear to be more genetically dissimilarity to that of *O. niloticus* injected with *O. aureus* DNA.

DISCUSSION

Interspecific hybridization was successfully obtained in many fish and shellfish genera and/or families as a means of improving production traits (Dunham et al., 2001; Hulata 2001). Hybridization between some species of tilapias such as Nile tilapia and Blue tilapia result in the production of predominantly male offspring (Hulata, 2001). This hybrid combines well the advantageous characteristics of both species, being more cold tolerant than *O. niloticus* and less borrowing in the mud than *O. aureus*. It also has good salinity tolerance and faster

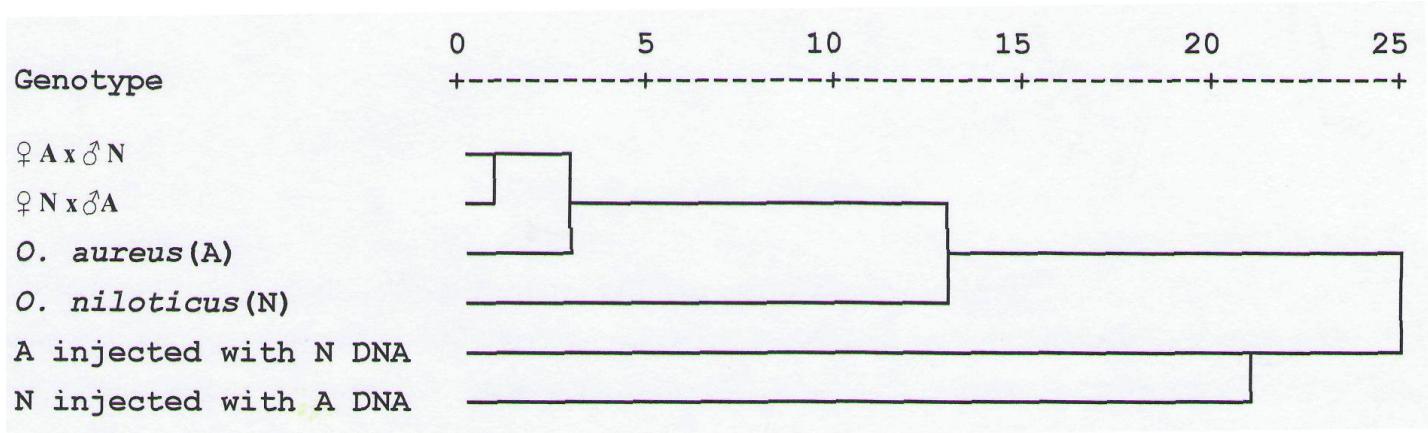


Figure 2. Dendrogram using average linkage (between groups) of the different genotypes based on RAPD fingerprinting as shown by hierarchical cluster analysis.

growth as a result of production of predominately male offspring, thus males grow faster than females in many tilapia (Hulata, 2001; Wohlfarth, 1994; Penman and McAndrew, 2000). The results of this study are consistent with these findings, thus the hybrid of $\text{♀ } O. niloticus \times \text{♂ } O. aureus$ and $\text{♀ } O. aureus \times \text{♂ } O. niloticus$ had significantly higher ($P \leq 0.05$) traits of growth performance and feed utilization than those of purebred of $O. niloticus$ and $O. aureus$.

When compared with the traditional approaches, genetically modified breeding avoids the productive isolation between two different species. Since more manipulated genes are available for foreign DNA transfer, it is hopeful for the investigators to shorten the breeding period through directional genetic breeding (Wang et al., 2001).

On the other hand, the success of the growth enhancement in this study with genetically modified fish is impressive and underscores their potential usefulness in aquaculture. Thus, genetically modified fish show a very good response, with more than 80 and 35% weight increase when compared with pure and hybrid fish, respectively. Most of the productive performance traits of genetically modified fish were improved significantly. In this connection, several studies reported that transgenic growth, body composition and feed utilization enhanced fish growth and show some improvements on both counts (Chatakondi et al., 1995; Rahman and Maclean 1999; Rahman et al., 1998; Maclean and Laight, 2000; Martinez et al., 2000; Devlin et al., 2004a, b; Kang and Devlin, 2003; Stevens and Devlin, 2000, 2005; Dunham et al., 2002; Raven et al., 2006; Hallerman et al., 2007; Oakes et al., 2007; El-Maremie, 2007 and El-Zaeem et al., 2011).

Genetically modified technology provides a means by which fish for human consumption could be raised to marked size in half of the normal time (Zbikowska, 2003). The phenotypic changes, such as increased growth rate,

are usually more prominent in the transgenic fish than those obtained by artificial selection or through efficient feeding regime (Sin, 1997).

Furthermore, the technique used in this study is concerned with the utilization of the whole gene, introns and exons and not only exons through mRNA and reverse transcriptase treatments (Ali, 2001). Thus, there is no need to utilize any kind of virus as the total DNA facilitates the introduction of foreign genes into cells with the aid of introns which act as retrotransposons (Hickey and Benkel, 1986).

In this connection, it was reported (El-Maremie, 2007; El-Zaeem et al., 2010, 2011) that a hypersaline genetically modified $O. niloticus$ with extraordinary growth rate can be produced by transfer of a foreign DNA isolated from sea bream and Artemia as a feasible and fast methodology when compared with interspecific hybridization. Genetically modified $O. niloticus$ treated with sea bream and Artemia DNA had surpassed growth rate under different levels of salinity up to 32 ppt, when compared with interspecific hybridization of $\text{♀ } O. niloticus \times \text{♂ } O. aureus$ and $\text{♀ } O. aureus \times \text{♂ } O. niloticus$ reared at the same levels of salinity. Genetically modified $O. niloticus$ that received Artemia DNA reared at 32 ppt of salinity had higher growth rate than that of genetically modified $O. niloticus$ treated with sea bream DNA at the same salinity level.

The results of this study suggested that genetically modified $O. niloticus$ and $O. aureus$ with higher growth rate can be produced using a feasible and fast methodology as compared to interspecific hybridization.

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