RAPD-MARKERS FOR DIFFERENT POPULATIONS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* L.) AND THEIR F_1 CROSSES

MOHAMED A. RASHED[1], HUSSEIN A. ELGHOBASHY[2], AMIN A. EL-GAMAL[3], ASHRAF A. RAMDAN[4] AND IBRAHEM H. IBRAHEM[2]

1. *Genetics Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*
2. *Genetics and Breeding Dept., Central Lab for Aquaculture Research (CLAR), Abbassa, Sharkia, Egypt*
3. *Animal Breeding Dept., Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*
4. *Hatchery Management and Reproductive physiology Dept., CLAR*

Abstract

Genomic DNA of five *Oreochromis niloticus* populations and their F_1 crosses were extracted from caudal fin tissues and examined for its quality. Six random primers (A07, A19, B05, B08, C03 and C16) were used to detect the genetic relationships among the bulked samples of the five founder populations. The phylogenetic tree showed that there were two main groups. The first one included Nasser Lake/Aswan populations and the second one comprised El-Qanater/El-Serw populations, while Kafr-ElSheikh population was branched from the second group to form a sub-group and was distant from the other populations. The data revealed that the highest means of weights (9.02g) and length (7.67cm) were for the crossing combinations of El-Serw as a female line with El-Qanater as a male line. On the other hand, the lowest means of weights (3.43g) and for lengths (5.44 cm) were for the cross of Kafr-ElSheikh as a female line with El-Qanater as a male line. Out of the 11 primers used in the present study, four (A06, A17, A19 and B08) were more effective than the others and successfully discriminated between the highest and the lowest F_1 crosses. The similarity indices average within the highest individuals line was (0.89), while the lowest individuals line showed a similarity average of (0.87). Polymorphism percentages ranged from 25 to 75% in the highest cross individuals and ranged from 0 to 93% in the lowest cross individuals. The RAPD primers which were used herein gave a reasonable diversity among the founder populations and between the highest and the lowest F_1 crosses.

Key Words: Nile tilapia, F_1 crosses, RAPD, Polymorphism

INTRODUCTION

Aquaculture is currently the fastest growing segment of food production in the world and by 2002 it was contributing approximately 30% by weight of fish and shellfish consumed worldwide (FAO, 2004). The value of farmed tilapia has witnessed a great increase during the past two decades, going from US$154 million in 1984 to US$1800.7 million in 2002 (El-Sayed, 2006). Nile tilapia (*Oreochromis niloticus*) is the most important cultured fish species in Africa and is farmed in a wide range of aquaculture systems (Pullin, 1985). Today, Nile tilapia represents more than 80% of the total tilapia production. The Egyptian Nile tilapia, *Oreochromis niloticus* (Class Pisces: Family Cichlidae), is considered as one of the major farmed freshwater fish. The prospects of genetic improvement of economically important traits are well documented in several fish species. For instance, for growth rate, genetic development gains around 10% of the mean per generation have been reported implying that growth rate can be doubled over a period of seven to eight generations (Gjedrem, 1997; Dunham *et al.,* 2001). During the last 15 years. The ease and simplicity of randomly amplified polymorphic DNA (RAPD) technique made it ideal for genetic mapping, plant and animal breeding programs and DNA fingerprinting, with particular utility in the field of population genetics (Ali *et al.,* 2004). Genetic variations can generate markers in many different types of studies in natural and cultured fish populations (Ferguson *et al.,* 1995). The development of RAPD markers, generated by the polymerase chain reaction technique (PCR), allows the examination of genomic variation without prior knowledge of DNA sequences (Hadrys *et al.,* 1992; Williams *et al.,* 1993). Several investigators examined DNA sequence polymorphism to detect any variations (Beaumont, 1994; Whitmore, 1990). Molecular markers are used as tools for estimating the phylogenetic relationships of different kinds of organisms (Avise, 1994). Three species of tilapia of genus *Oreochromis* and four subspecies of *O. niloticus* were analyzed using RAPD technique. Bardakci and Skibinski (1994) reported that thirteen decamer primers were used to detect polymorphisms within

and between populations. The results showed different RAPD fragment patterns for different species, although not always for different subspecies. The dendrogram linked *O. mossambicus* with the subspecies of *O. niloticus*, with *O. aureus* as out-group, and confirmed that RAPD markers might be useful for systematic investigation at the level of species and subspecies. RAPD polymorphisms are inherited in a Mendelian fashion and can be used as genetic markers. Also it notable that there were differences detected among DNA fingerprint patterns of mixed DNA samples from five rainbow trout and two cutthroat trout strains. DNA fingerprints of mixed DNA samples can be useful for assessing relationships between closely related populations due to the high level of genetic differentiation which was detected by this method. It was suggested that the mixing approach can be particularly useful in breeding programs (Palti *et al.,* 1997). Iturra *et al.* (1998) used RAPD assay and bulked segregant analysis technique to identify and generate polymorphic bands which were amplified preferentially in males of the strains of rainbow trout. The genetic variations among three populations of *O. niloticus* from different hatcheries in Egypt (Sohag, Voki, and Manzalla) were estimated among and within populations. The results indicated that Voki population has the highest homogeneity value because it was subjected to inbreeding scheme for several generations. The study also concluded that the molecular markers have an ability to trace the genetic variability (Rashed *et al.,* 1998). On the other hand, Bardakci (2000) used three sets (OPA, OPB and OPC) of randomly amplified polymorphic DNA (RAPD) markers in sex discrimination in Nile tilapia (*Oreochromis niloticus*) to develop sex-linked RAPD markers, with three different genotypes of Nile tilapia; XX female, XY male and YY male were used. Comparison of amplification of pooled DNA samples from these three genotypic sexes failed to show any reproducible and clear-cut RAPD markers occurring in only one sex. Likewise, Povh *et al.* (2005) applied the RAPD markers to estimate the genetic divergence and variability of the Nile tilapia strains (*Oreochromis niloticus*) and concluded that genetic variability estimation is highly important in order to achieve genetic improvement. Ibrahem *et al.* (2007) studied the

phenotypic variability of live weights of five different sources; Kafr-ElSheikh, El-Serw, El-Qanater, Aswan and Nasser Lake in order to select the parent stock from the base population. Nine different crosses based on the sources of *Oreochromis niloticus* populations which were carried out to identify the best cross combinations for weight and length traits. The latter authors showed that the combinations that included El-Serw population as female gave a high growth rate.

The aims of the present study were to detect the genetic variations among collected *Oreochromis niloticus* stocks and to detect the genetic relationship between the highest and the lowest F_1 crosses using RAPD markers for important traits in *Oreochromis niloticus.*

MATERIALS AND METHODS

Fish Collection and Crossing

Batches of fish were collected from five different geographic sources {Kafr-ElSheikh (Kf), El-Serw (S), El-Qanater (Q), Aswan (A) and Nasser Lake (N)} to obtain as much diversity as possible through matings between and within all collected sources. Nine different crosses based on five sources of *Oreochromis niloticus* populations were carried out to identify the best cross combinations for weight and length traits. The best crossing combination for fish weights and lengths of F_1 hybrids which was carried out in a separately hapas for rearing and growing was evaluated. Therefore, three females to one male were randomly chosen were kept in circulating fiber ponds for hatching (Table 1). Fish fry (~0.1g) that were produced from each crossing combination were raised in two separate hapas (1 x 1 x 1.5 m) as in two replicating after two weeks of hatching. After the growing period (10 weeks), 50 fish individuals were collected from each hapas to measure their weights and lengths.

Table 1. The Nine randomly mating systems according to the sources of Nile tilapia

		Males ♂♂ sources			
Females ♀♀ sources		El-Serw (S)	Nasser Lake (N)	El- Qanater (Q)	Aswan (A)
	El-Serw (S)	NA*	3	5	7
	Kafr-ElSheikh (Kf)	1	NA	6	8
	El-Qanater (Q)	2	4	NA	9

NA* = not available

Samples preparation

Fish samples were placed directly in ultra low temperature freezer at -70°C until they were dissected. Small piece of fresh caudal fin tissue (1cm) was cut and put in eppendorf tube containing a 70% isopropanol alcohol and keep at 4 °C until use. Very small piece of kept caudal fin tissue (3 x 3 mm) was cut and put in order on soft papers until dry, then transferred to a new marked eppendorf tube. RAPD-PCR technique of random genomic DNA using random primers (Callejas and Ochando, 1998) was used as shown in Table (2).

Genomic DNA extraction from parents and F_1

Ten samples per each founder population and 15 fish of F_1 individuals for higher and lower lines were collected and their genomic DNA were extracted from caudal fin tissues according to the salting out procedure (Miller *et al.* 1988). The method was carried out at the Molecular Genetic Laboratory, Faculty of Agriculture, Ain Shams University, Egypt. Extracted genomic DNA gave a purity ratio of DNA to proteins in a range of 1.7 to 1.9 with a concentration of 100ng/µl when analyzed by spectrophotometer ~85%. After checking the genomic DNA on a 0.7% agarose gel, each ten DNA samples from each founder population were mixed in bulked sample for PCR amplification (Lukyanov *et al.,* 1996). PCR amplification for F_1 hybrids were carried out individually. A set of thirteen decamer primers (10 nucleotides for each primer) from Operon (OP) Technologies Co. was used in this

work as shown in Table (2). The PCR mixture and amplification conditions were prepared according to Williams *et al.* (1990).

PCR conditions

The total volume (12.5 µl) of PCR mixture per genotype consisted of 2.5mM dNTPs (1.3 µl), 10X reaction buffer (1.5 µl MgCl2), 15 ng primer (1.0 µl), 25 ng template DNA (1.0 µl), 5 units of Taq super thermal (0.2 µl) and 7.5 µl H2O (d.d.w). Primus Thermocycler was used for DNA amplification and programmed as follows; Denaturing (one cycle) at 94°C for 4 minutes; Annealing (35 cycles) at 94°C for 45 second, 37°C for 45 sec and 72°C (initial extension) for 45 sec; Extension (one cycle) at 72°C for 10 min; and Store at 4°C for 10 min.

Table 2. The thirteen operon random primers and their sequences used for the detection of polymorphism in Nile tilapia populations.

Primers (OP)	Sequence (5` to 3`)	GC Content (%)	Primers (OP)	Sequence (5` to 3`)	GC Content (%)
A06	GGT CCC TGAC	70	B08	GTC CAC ACG G	70
A07	GAA ACG GGTG	60	C03	GGG GGT CTT T	60
A17	GAC CGC TTGT	60	C13	AAG CCT CGT C	60
A19	CAA ACG TCGG	60	C15	GAC GGA TCA G	60
B01	GTT TCG CTCC	60	C16	CAC ACT CCA G	60
B03	CAT CCC CCTG	70	C17	TTC CCC CCA G	70
B05	TGC GCC CTTC	70			

OP; Operon Technologies Co.

Data analysis for RAPD-PCR

Genetic variability was estimated by calculating percentage the polymorphic loci. Each individual was scored for the presence (1) or absence of (0) amplified products. Accordingly, relative molecular size of each fragment could be measured and scored manually and by GelAnalyzer Version 3, program software (2007). The statistical analysis was done for each primer separately then for the

combined data for all primers. Data were entered into a binary matrix and pairwise similarity indices were constructed (Lynch, 1990; Sneath and Sokal, 1973). The data generated from pattern of RAPD patterns were introduced into SPSS version 10 package program (DICE method) according to binary values. The output results involved both different unweighted pair-group method of analysis (UPGMA) was used to construct the dendrogram tree, according to Sneath and Sokal (1973).

RESULTS AND DISCUSSION

DNA polymorphisms

Genomic DNA of the five different stock populations and their F_1 crosses were extracted from caudal fin tissues and analyzed in order to detect molecular genetic variations for *O. niloticus* parents and their F_1 crosses. The highest means of weights (9.02g) and lengths (7.67cm) were obtained from the cross between of El-Serw as a female line (S ♀) with El-Qanater as a male line (Q ♂). On the other hand, the lowest means for weights (3.43g) and for lengths (5.44 cm) were for the cross of Kafr-ElSheikh as a female line (Kf ♀) with El-Qanater as a male line (Q ♂). Therefore, it was important to explore the genetic characterization of these crosses.

RAPD-markers among the founder populations

Bulked samples were chosen from each population to study DNA polymorphisms and to construct the dendrogram among the five *O. niloticus* populations, (Q, S, Kf, N & A). The determination of genetic diversity based on 71 RAPD fragments by six-random primers among these founder-populations is shown in Table (3). Six random primers (A07, A19, B05, B08, C03 & C16) were used to detect the genetic relationships among the bulked samples of the five founder populations (Fig. 1). The similarity index values of 0.78, 0.76, 0.73 & 0.69 were estimated between Kafr-ElSheikh (Kf) population with Nasser Lake (N), El-Serw (S), El-Qanater (Q) and Aswan (A) populations, respectively. The highest similarity index value (80%) was noticed between Nasser Lake and Aswan populations and

between El-Serw and El-Qanater populations. While the lowest value (69%) was observed between El-Serw and Nasser Lake populations and between Kafr-ElSheikh and Aswan populations. The genetic relationships tree resulted from cluster analysis is illustrated in Figure (2). It is noticed that, there were two main clusters, the first one included Nasser Lake and Aswan populations and the second one comprised El-Qanater and El-Serw populations, while Kafr-ElSheikh population was branched from the second cluster to form a sub-cluster and was distant from the other populations.

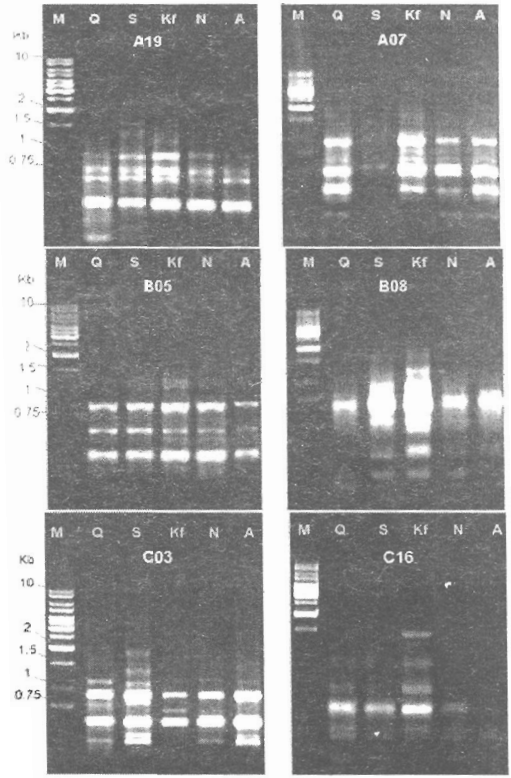

Figure 1. Bulked-DNA polymorphisms among the five populations (Q, S, Kf, N & A) using RAPD bands generated by six polymorphic random primers, M=DNA marker.

Table 3. The similarity index values among the five populations based on results of six polymorphic random primers; A07, A19, B05, B08, C03 and C16.

Population	Similarity indices			
	Q	S	Kf	N
S	0.80			
Kf	0.73	0.76		
N	0.79	0.69	0.78	
A	0.71	0.71	0.69	0.80

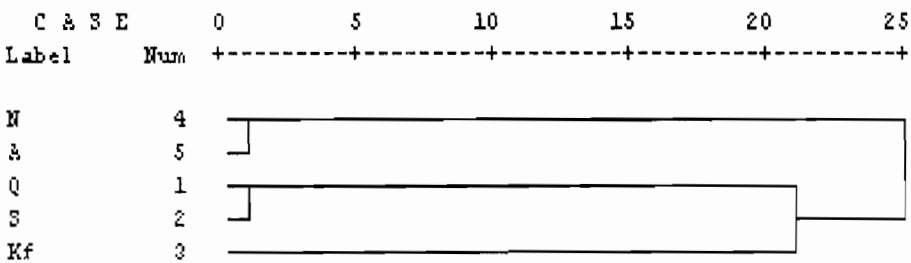

Figure 2. Genetic relationships tree using UPGMA among the five populations generated by six polymorphic random primers.

The results suggested that molecular analysis can be applied to aquaculture genetics including assessment of inbreeding rates and mixed cultured fish by genetic markers to identify individuals and population groups according to Harris *et al.* (1991) and Sandlund *et al.* (1992). Likewise, this result agreed with the conclusion of Palti *et al.* (1997). The latter authors reported that DNA fingerprints of mixed DNA samples can be useful for assessing relationships between closely related populations due to the high level of genetic differentiation detected by this method. It was further suggested that mixing (bulked) approach could be particularly useful in breeding programs. In contrast, years of domestication may

allow inbreeding or genetic drift to cause a reduction in the genetic diversity of these populations. These findings were almost in agreement with Hulata *et al.* (1986) who reported that the genetic bottlenecks and a high level of inbreeding may have significantly limited the genetic variation and marginal response to genetic improvement by the starting of the experiments, especially when a small founder population was used. So, the effective management of fisheries or fish farms requires the ability to discriminate among stocks. One needs to identify non-interbreeding populations to assess the gene flow between different genetic stocks and to monitor temporal changes in the gene pools (Rico *et al.,* 1997). Also the influence of female pooling in the crossing may affected the important growth traits (Ibrahem *et al.,* 2007). The latter authors studied nine different crosses based on the five sources of *Oreochromis niloticus* populations and reported that based on F_1 combinations of Nile tilapia hybrids, El-Serw female line resulted in high growth than other sources.

RAPD-markers among F_1 crosses

The 11 random primers; A06, A17, A19, B01, B03, B08, C03, C13, C15, C16 and C17 were used to discriminate between 15 individuals from the highest and from the lowest crosses in weight and length as shown in Figures (3 to 5). Using these 11 primers, 89 and 102 RAPD fragments were produced in the individuals of the highest and the lowest crosses for weight and length traits, respectively. The results of the highest and the lowest F_1 crosses are shown in Table (4). The similarity index average within the highest cross individuals was 0.89, while the lowest cross individuals showed a similarity average of 0.87. Polymorphism percentages were ranging from 25 to 75% in the individuals of the highest cross and from 0 to 93% in the individuals of the lowest cross.

It can be concluded from Figures (3 to 5) for RAPD profiles in F_1 and Table (5) with the 11 primers that four of RAPD primers namely; A06, A17, A19 and B08 were more effective and successful to discriminate between the highest and the lowest F_1 crosses. While the rest were less effective to discriminate them. As shown in Table (5), no marker was detected in the lowest cross individuals for

B01primer, while the same primer showed two marker bands in the highest cross individuals with molecular sizes of 1800 and 1125 bp. On contrary, primers C16 and C17 showed one marker band with molecular size around 363 bp for primer C16 and 942 bp for primer C17 in the lowest cross individuals, while both primers did not show any marker band in the highest cross individuals. Only one marker band was detected in both the highest and lowest crosses for primers B03, C03 and C13 with molecular size of 183 and 511bp for primer B03, 249 and 1084 bp for primer C03 and 876 and 787 bp for primer C13 (Table 5). Primers A06, A17, A19 and B08 showed more marker fragments in the individuals of the both groups. With primer A06, two and six marker bands were observed with fragment sizes of (698 and 380bp) and (3070, 2779, 1788, 1489, 1376 & 1063 bp) in both the highest and the lowest crosses, respectively. For the A17 primer, four and two marker bands were detected with fragment sizes of (957, 808, 573 & 444 bp) for the highest cross and (886 and 188 bp) for the lowest cross. With A19 primer, two marker bands (511 and 192 bp) for the highest cross and five ones (1022, 920, 323, 285 and 174 bp) for the lowest cross were observed. Moreover, five and six marker bands were observed by primer B08 for the highest cross and the lowest cross, respectively. The results also revealed that the primer C15 showed two marker bands in the highest cross with fragment size of 436 and 303 bp and one marker band in the lowest cross with fragment size of 211 bp as shown in Table (5). Therefore, the A06, A17, A19 and B08 which are considered as effective and successful primers in discriminating between the highest and the lowest crosses for growth traits in Nile tilapia.

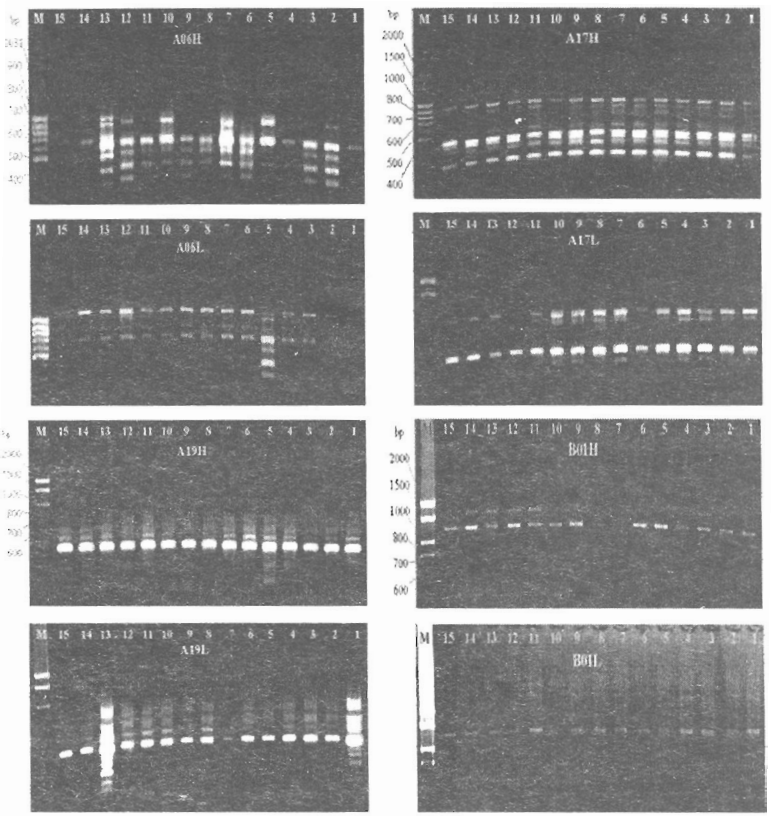

Figure 3. DNA polymorphism generated by random primers A06, A17, A19 and B01 for fifteen individuals of the highest cross (H) and the lowest cross (L), M= DNA marker.

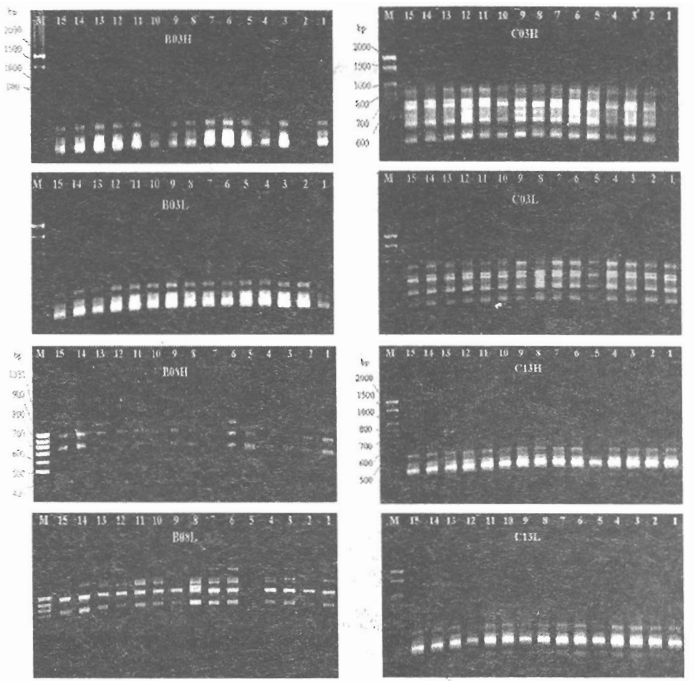

Figure 4. DNA polymorphism generated by random primers B03, B08, C03 and C13 for fifteen individuals of the highest cross, (H) and the lowest cross, (L), M= DNA marker.

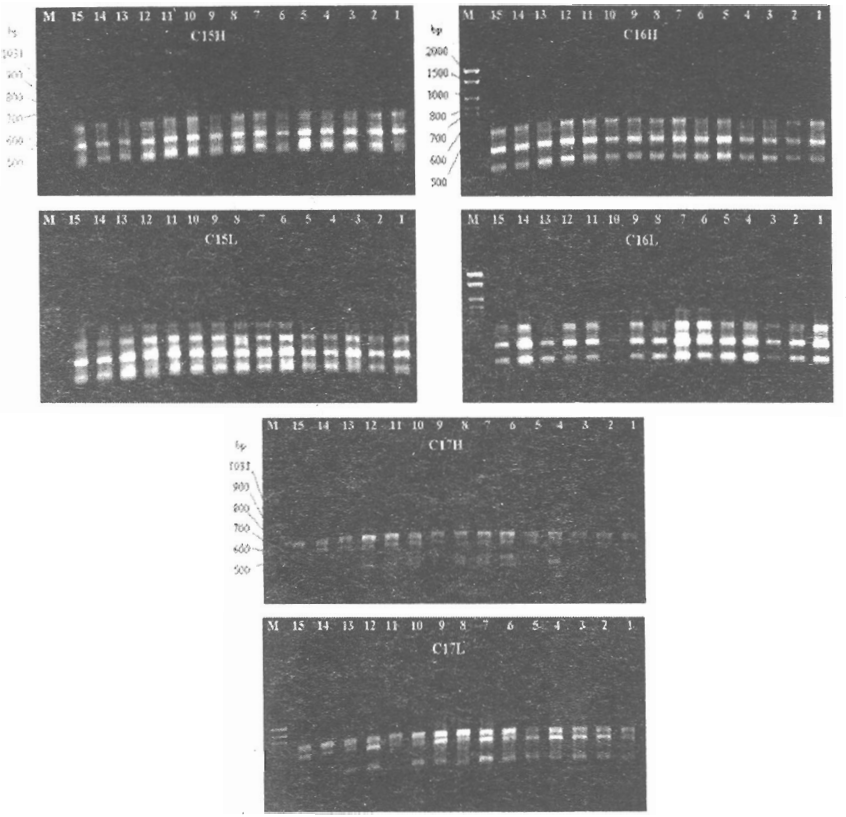

Figure 5. DNA polymorphism generated by random primers C15, C16 and C17 for fifteen individuals of the highest cross (H) and the lowest cross (L), M= DNA marker.

Table 4. The total number of detected, polymorphic, monomorphic and unique bands and DNA polymorphisms within the highest and the lowest crosses generated by 11 random primers.

	Highest cross					Lowest cross				
Primer	TB	Poly. b	Mono. b	Unq. b	P%	TB	Poly. b	Mono. b	Unq. b	P%
A06	10	6	4	ND*	60	14	12	2	ND	86
A17	12	5	6	1	50	11	8	3	ND	73
A19	10	5	5	ND	50	15	14	1	ND	93
B01	4	3	1	ND	75	2	1	1	ND	50
B03	4	1	3	ND	25	4	0	4	ND	0
B08	8	5	3	ND	63	11	6	4	1	64
C03	10	7	3	ND	70	12	6	6	ND	50
C13	9	4	5	ND	44	10	4	5	1	50
C15	7	2	5	ND	29	7	0	6	1	14
C16	7	2	4	ND	29	7	4	3	ND	57
C17	8	6	2	ND	75	9	3	6	ND	33

ND*= not detected, TB= total band numbers, poly. b = polymorphic bands, mono. b= monomorphic bands, unq. b= unique band and P%= DNA polymorphism percentage

Table 5. RAPD-marker bands for the highest (H) and the lowest (L) cross combinations detected by different random primers.

	H. Cross		L. Cross	
Primer	No. marker bands	Fragment size (bp)	No. marker bands	Fragment size (bp)
A06	2	~698, 380	6	~3070, 2779, 1788, 1489, 1376, 1063
A17	4	~957, 808, 573, 444	2	~886, 188
A19	2	~511, 192	5	~1022, 920, 323, 285, 174
B01	2	~1800, 1125	0	—
B03	1	~183	1	~511
B08	5	~1839, 1706, 1384, 1120, 652	6	~2097, 1918, 1637, 1555, 1452, 1239
C03	1	~249	1	~1084
C13	1	~876	1	~787
C15	2	~436, 303	1	~211
C16	0	—	1	~363
C17	0	—	1	~942

Generally, RAPD markers may provide a powerful tool for determining the occurrence and extent of hybridization. Molecular markers typically possess simple modes of expression and inheritance and more likely than morphological measurements to provide evidence of low levels of hybridization (Gottlieb, 1981; Tanksley and Orton, 1983; Nason *et al.,* 1992). The most striking observation from the aforementioned results of RAPD-PCR is that the RAPD primers which were used herein gave a reasonable diversity within and among the founder populations and between the highest and the lowest F_1 crosses. The present study had restricted on the farmed Nile tilapia populations to evaluate the weight and length traits as indices of phenotypic performance. Besides, the RAPD markers were used herein to detect the minor or wide polymorphisms in both the parents and their F_1 hybrids. No wide spatial variations were detected among the five applied populations which were collected from different farms in Egypt, so no highly polymorphism was found using RAPD primers. The results herein indicated that there was a moderate polymorphism which allow introduction of these populations in hybridization programs to obtain the best cross combination for traits under study. The results of this study agreed with the results of Liu *et al.* (1999) who tested the efficiency of 100 DNA random primers to identify DNA-based genetic polymorphism for constructing a genetic linkage map of both channel catfish and blue catfish. Low levels of intra-specific variation (within) in RAPD profiles within strains were found. Besides, high levels of inter-specific variation (between) were detected between channel catfish and blue catfish. The RAPD markers were highly reproducible in a size range from 200 to 1500 bp. In contrast, no differences in RAPD profiles between F_1 hybrids were found unlike the current study which found RAPD polymorphisms between the highest and the lowest F_1 crosses (Tables 4 & 5). The latter authors concluded that the RAPD is perhaps the most appropriate and economical tool for hybrid identification because of its dominant inheritance. The current study agreed with the conclusion which was confirmed by many authors who reported that molecular identification of hybrids is much more effective, as hybrids are not necessarily intermediate forms and may exhibit novel

morphologies outside the range of the parental lines (Crapon de Caprona and Fritzsch, 1984; McElroy and Kornfield, 1993). The fingerprints generated by the used primers herein revealed different unique profiles for each population in terms of number and position of RAPD bands. Genetic similarity indices (Dice method) and UPGMA clustering were used to construct the genetic relationships based on RAPD bands among Nile tilapia populations. So, the present study provides evidence that the RAPD markers can be effectively used to discriminate among Nile tilapia populations and their crosses which agreed with Bardakci and Skibinski (1994) who used RAPD method to detect variation among populations, sub-populations and species. In this regard, the information on the genetic structure of farmed fish species is essential for studying molecular systematic and optimizing fisheries management and fish farming (Barman *et al.,* 2003). The RAPD assay in the current study was evaluated for studying relationships and diversities especially for farmed tilapia population. Likewise, Hassanien *et al.* (2004) detected genetic diversity using RAPDs among different *O. niloticus* populations in Egypt (Nile River: Cairo, Assuit and Qena and Delta Lakes: Borollus and Manzalla). From which, the population from Qena had the highest degree of polymorphism.

In conclusion, the genetic variability in fish populations depends mainly on two approaches; biometric measurements and biochemical and molecular genetic markers. Besides, the environmental influences play a role in this variation and performance of genetic and physiological traits. Therefore, genetic variability is an important feature of populations at the level of short term fitness of individuals and at the level of long term survival of the population. In addition, the genetic variation allows adaptation to changeable environmental conditions. It is similarly important in farmed populations since it concentrates on selective breeding and prevents loss of fitness due to inbreeding depression. Moreover, introduction of the natural or feral sources into hybridization program may be more useful to enhance the genetic improvement for the most important traits in Nile tilapia. Hence, more variations in the sources of population lead to more significant differences in the traits under study.

AKNOWLEDGMENT

This study was funded by the project of producing pure lines of Egyptian Nile tilapia using molecular genetic techniques and selection from the Academy of Scientific Research and Technology, Ministry of Scientific Research, Egypt. We are grateful thanks to the many collaborators and researchers of this work through collaboration between the Genetics and Animal Production Departments, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.

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الدلائل الوراثية الجزيئية لعشائر مختلفة من البلطي النيلي وهجن الجيل الأول

محمد عبد السلام راشد[1] ، حسين عطية الغباشى[2] ، أمين عبد المعطى الجمل[3] ، أشرف عبد الرحمن رمضان [4]، إبراهيم حسن إبراهيم[2]

١. *قسم الوراثة – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة.*
٢. *قسم الوراثة والتربية – المعمل المركزي لبحوث الثروة السمكية بالعباسة – أبو حماد– الشرقية.*
٣. *قسم الإنتاج الحيواني – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة.*
٤. *قسم التفريخ وفسيولوجيا التكاثر– المعمل المركزي لبحوث الثروة السمكية بالعباسة – أبو حماد– الشرقية*

هدفت الدراسة إلى تقييم التباين الوراثي لخمسة عشائر من البلطي النيلي (كفر الشيخ، السرو، القناطر، أسوان، بحيرة ناصر) والمجمعة من مصادر مختلفة على مستوى الأدلة الجزيئية باستخدام تقنية الـ RAPD-PCR لرسم شجرة القرابة والتشابه بين العشائر الأبوية الخمسة. كما تم تقييم الجيل الأول الهجين F_1 من خلال تقدير التباين الوراثي داخل العشائر وبينها باستخدام نواتج التفريد الكهربى لـ (RAPD-PCR) مع بادئات عشوائية التتابع (Random primers). حيث استخدمت الأدلة الوراثية الجزيئية RAPD-markers والمميزة للتقييم بين أفراد أعلى وأقل مجموعة في صفتي الوزن والطول من هجن الجيل الأول F_1crosses.

تم استخلاص الحامض النووي (DNA) من أنسجة الزعنفة الزيلية للخمسة عشائر وهجن الجيل الأول ، واستخدمت تقنية الـ (RAPD-PGR) للكشف عن التباينات الوراثية على المستوى الجزيئي لكلا من الآباء وهجن الجيل الأول لأسماك البلطي النيلي.

حيث استخدمت التقنية على مخلوط الأحماض النووية Bulked DNA لكل عشيرة من الآباء للكشف عن الاختلافات فيما بينها على مستوى الـ DNA ، وقد نجحت ستة بادئات، (A07, A19, B05, B08, C03, C16) في التمييز بين هذه العشائر وتحديد درجة التشابه والاختلاف فيما بينها. وقد أشارت النتائج الخاصة بشجرة العلاقة الوراثية إلي تفرع الآباء إلي مجموعتين رئيسيتين، الأولى ضمت عشيرة أسوان وبحيرة ناصر كمجموعة واحدة، في حين

كونت عشيرة السرو والقناطر المجموعة الثانية التي تفرعت منها عشيرة كفـر الشـيخ لتكـون مجموعة فرعية حيث كانت ذات علاقة تشابه أقل بالنسبة لباقي العشائر.

أظهرت أفراد الجيل الأول الهجين الناتجة من التزاوج (إناث السرو x ذكور القناطر) أعلى متوسط لصفتي الوزن والطول بينما أظهرت أفراد الجيل الأول الهجين الناتجة من التزاوج (إناث كفرالشيخ x ذكور القناطر) أقل متوسط بالنسبة لصفتي الوزن والطول ، لذا كان لابد من الكشف الجزيئي على مستوى الـ DNA لهذه الهجن، واستخدم لهذا التحليل ١١ بادىء (A06, A17, A19, B01, B03, B08, C03, C13, C15, C16 and C17) للتفريق بين أفراد أعلى وأقل هجين. وقد تم تحديد بعض الدلائل أو العلامات الجزيئية للمجموعة الأعلى والأقل في صفتي الوزن و الطول ومنها تم تحديد الاختلافات الوراثية للمجموعتين. وقد ساهمت تقنية (RAPD-PCR) في تحديد الاختلافات الوراثية بين وداخل العشائر المستخدمة في التربية.

وقد أوضحت النتائج المتحصل عليها من هذه الدراسة أن البادئات الأربعة A6 وA17 و A19 وB08 كانت الأفضل في التمييز بين الهجين العالي والهجين المنخفض. تم أيضا حساب النسبة المئوية للتباين الوراثي والتي تراوحت من ٢٥ إلي ٧٥% في الهجين العالي بينما تراوحت النسبة بين الصفر إلي ٩٣% في الهجين المنخفض. ومن ذلك تبين أن استخدام تقنية الـ RAPD-PCR قد أعطت هنا اختلافات نوعية بين عشائر الآباء وبعضها وبين هجن الجيل الأول العالي والمنخفض وساعدت بقدرة وكفاءة مناسبة في كشف التباينات الوراثية بين العشائر المدروسة ، حيث اكتشفت دلائل وراثية جزيئية خاصة بكل عشيرة ودلائل أخرى خاصة بالمجموعتين (الأعلى والأقل) في صفتي الوزن والطول لأفراد الجيل الأول الهجين.