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Genetic Variations and Inheritance of Some DNA Markers in Three Constructed *Oreochromis niloticus* Families

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Abstract: The current study aimed to develop DNA markers in three constructed *O.niloticus* families (I, II and III) for high growth rate performance. Seven SSR and 11 RAPD primers were used and their loci were estimated. All studied SSR loci were polymorphic. Inheritances of some DNA markers from parents to their offspring were determined. The overall gene diversity (h) based on SSR data was higher than based on RAPD data. Some RAPD and SSR markers were determined and were found to be associated with fish growth rate performance. Genetic markers developed in this study will play a valuable role in maintaining and monitoring future husbandry practices undertaken during the culture of the applied fish families in our farms.

Key words: *Oreochromis niloticus* · DNA markers · Family selection

INTRODUCTION

The future of Tilapia stock (as a major international commodity fish) improvement will rely on appropriate stock choice, development of sound management techniques and selective breeding. The basis of this approach is the ability to characterize and monitor tilapia genetic resources under culture, provide a sound knowledge of the genetic characteristics of each stock and to examine the effects of management practices on the gene pools of each stock [1].

Development of local fish breeds are important because many of them utilize lower quality feed, are more resilient to climatic stress and represent a unique source of genes for improving health [2]. In addition, studying the relative contribution of each fish parent to the next generation is a great concern, especially when some of the offspring are intended to be used as parents of the subsequent generation [3].

A group of Tilapia genetic projects such as (GIFT), (GMT/YY-supermale), (IDRC) and (SEAFDEC) were designed for improving of some economic characters in the Nile tilapia in many countries, especially in Asia [4]. Some products of these projects were developed and field-tested such as SEAFDEC [5]. The genetic structure of these improved products should be characterized to effectively manage and conserve their economic characters. In addition, molecular genetic markers were widely used to identify lines or strains, define stock diversity, monitor inbreeding, diagnose simply inherited traits and even improve stocks [6, 7]. The application of DNA-based genetic analysis (as marker assisted selection) in Tilapia research and stock development and management is still not fully maximized [7, 8].

The present study aimed to estimate some growth rate characters and to identify some molecular genetic features for the highest and lowest crossing combinations of three constructed *O.niloticus* families.

MATERIALS AND METHODS

Fish: The fish parent lines were obtained from PNGS project products [9] during the year 2006. An individual mating experiment was designed and carried out according to PNGS project at the Fish Experimental Station of the Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt during the year 2006. One female (S line) to one male (L1, L2 and L3 lines) were kept in 1m^3 PVC pond with 0.75 m^3 fresh water.

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The Individual Mating Experiment Was as Follow: Family I: ♀ (S) x ♂ (L1), Family II: ♀ (S) x ♂ (L2) and Family III: ♀ (S) x ♂ (L3). The F1 hybrids generated from each cross (0.1g average weight) was reared and evaluated in a separately hapa (1x1x1.5m) as described in PNGS project [9].

Spawning, rearing and collections of fry and growth rate were measured according to PNGS [9].Weights and lengths of 100 individual fish of each progeny of the three crosses were recorded biweekly for 10 weeks. The condition factor of individual fish, as an index of growth rate, was calculated as described by Bagenal and Tesch [10].

Molecular Marker Analysis: DNA extraction was performed for 15 fish individuals for the three families according to Hillis *et al.* [11].

RAPD Analysis: Eleven RAPD primers (Operon Technologies, Inc.; Alameda, California, EUA) were used which scoreable amplified bands. The primer codes were OPA1, OPA7, OPA11, OPA13, OPA19, OPB8, OPC3, OPC6, OPC8, OPC12 and OPC20. PCR mixture, reaction conditions and product separation were carried out as described by Rashed *et al.* [7].

SSR Analysis: To survey genetic variation in the three fish families, seven microsatellite primers were originally selected which developed by Lee and Kocher [12]. PCR mixture, reaction conditions and product separation were carried out as described by Ibrahim [13] with minor modifications. The annealing temperatures were (53.9°C, 55.2°C), (51.7°C, 55.7°C), (52°C, 54.2°C), (49.7°C, 51.1°C), (49.7°C, 51.1°C), (49.7°C, 51.1°C) and (49.7°C, 51.1°C) for GM 538 (F, R), GM 531(F, R), GM 211(F, R), UNH185 (F, R), UNH136 (F, R), UNH142 (F, R) and UNH1004 (F, R), respectively.

Statistical Analysis: All gels were analyzed using Total Lab program v2.01 as described by Rashed *et al.* [7]. These data were analyzed as diploid data for Dominant (RAPD) and Co-dominant (SSR) markers by standard POPGENE program (version 1.32), for population genetic analysis [14].

RESULTS

Performance Variation: The mean and standard error for weight, length and condition factor for each of the three crosses are presented in Table 1.

Concerning the studied characters, the highest mean values were revealed from Family (I) progeny, while the lowest values were revealed from Family (III) progeny. The mean values of condition factor for all families were less than 2 which reflected the good conditions of the resulted fish families (Table 1).

Genetic Variations:

Genetic Variations Based on RAPD Analysis: The number of amplified fragments (bands), number of polymorphic fragments and its frequencies generated by each primer were scored. The number of amplified fragments were ranged from 3 to 16 and the number of polymorphic fragments were ranged from 0 to 15 (Table 2).

Genetic Variations Within Each Family: The percentage of polymorphic bands generated by each primer within each family was calculated. They were 30.0, 46.25 and 30.0% for families I, II and III, respectively (Table 3). The number of amplified fragments was ranged from 3 to 8 within each family.

Out of 11 used RAPD primers, only two primers showed no polymorphic bands (C12 and A13). On the other hand, the other nine primers generated from 2 to 5, 1 to 11 and 1 to 5 fragments in families I, II and III, respectively.

The current results presented three family-specific RAPD markers. One of them was in family II using primer A1 at fragment size of 121bp. The other two markers were in family I at fragment size of 247 and 1025 bp which generated by C20 and C3 primers, respectively (Table 2). The mean of observed number of alleles (na), Effective number of alleles (ne), Nei's gene diversity (h) and Shannon's Information index (I) within each family were calculated (Table 3). Family II had the highest values for the previous estimates.

Genetic Variations among Families: The genetic variation values among the applied fish families were calculated (as a genetic differentiation). These were 0.028, 0.025 and 0.029 between (I-II), (I-III) and (II-III) family pairs, respectively as shown in Table 5.

Genetic Variations Based on SSR Analysis: Generally, all studied loci were polymorphic. Each one of the SSR fragments, with different molecular size bp, was considered as an allele. The molecular weight of each studied allele was estimated. The ranges of these molecular sizes were (178 to398), (178 to 216), (174 to 220),(166 to 350), (124 to 198), (160 to 230) and (200 to 320) for GM211, UNH136, UNH142, UNH1004, UNH185, GM 538 and GM 531 primers, respectively (Table 2).

Table 1: Mean and standard error (SE) for fish weight, length and condition factor characters of the three crosses

		Weight		Length		Condition Factor	
S♀♀	Male ♂♂	Mean	SE	Mean	SE	Mean	SE
	Q	9.02	0.47	7.70	0.13	1.8	0.01
	A	8.46	0.45	7.50	0.14	1.8	0.01
	N	7.51	0.49	7.26	0.16	1.7	0.02

Table 2: Locus code, detected bands, polymorphic alleles (bands), range of fragment sizes and average of band frequencies generated by RAPD and SSR markers

			RAPD						SSR		
Code	DB	PB	R FS (Kb) for PB	Fsm (bp)	ABF	Code	DB	PB	R FS bp	Fsm(bp)	ABF
C12	5	0	-	-	1	GM211	6	6	178 -398	298-398	0.32
C6	16	15	0.24-0.008	-	0.31	unh136	2	2	178- 216	-	0.56
A13	3	0	-	-	1	unh142	4	4	174 - 220	-	0.5
A1	6	1	0.121	121	0.87	unh1004	7	7	166 - 350	-	0.26
C20	9	7	0.340 - 0.118	247	0.49	UNH185	2	2	124 - 198	-	1
C8	10	8	0.491 - 0.279	-	0.39	GM538	4	4	160 - 230	-	0.5
B8	5	2	0.353 -0.245	-	0.86	GM531	5	5	200 - 320	-	0.34
A7	8	6	1.277- 0.297	-	0.83						
A19	6	5	1.125- 0.535	-	0.88						
C3	6	4	1.656- 0.972	1025	0.63						
A11	6	6	1.061- 0.570	-	0.39						

DB= number of detected bands, PB= polymorphic bands, ABF= average of band frequencies,
fsm= family specific DNA markers and R FS= range of fragment size

Table 3: Mean±SE of Actual number of alleles (na), Effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I) and percentage of polymorphic loci (%PL) for each studied family

	RAPD				SSR			
	Family I	Family II	Family III	Total	Family I	Family II	Family III	Total
na	1.3±0.1	1.46±0.2	1.3±0.1	1.6±0.1	2.7±0.04	2.57±0. 04	2.14±0.1	4.14±0.6
ne	1.21±0.04	1.31±0.1	1.2±0.1	1.3±0.1	2.03±0.2	2.04±0.2	1.96±0.04	2.6±0.2
h	0.11±0.04	0.176±0.08	0.12±0.04	0.188±0.04	0.46±0.08	0.46±0.08	0.48±0.3	0.55±0.05
I	0.17±0.08	0.259±0.08	0.17±0.08	0.293±0.08	0.76±0.1	0.74±0.1	0.69±0.04	1.01±0.1
%PL	30	46.2	30		85.7	85.7	100	
Mean Fst.				0.268				0.149

Fst=Genetic differentiation

Table 4: Summary of Heterozygosity statistics for all SSR Loci within each studied family

	Family I			FamilyII			FamilyIII		
Locus	Obs_Het	Exp_Het	Ave_Het	Obs_Het	Exp_Het	Ave_Het	Obs_Het	Exp_Het	Ave_Het
GM211	1	0.644	0.58	0.80	0.64	0.58	1	0.64	0.58
unh136	0	0	0.106	0	0	0.106	0.40	0.35	0.106
unh142	1	0.55	0.50	1	0.55	0.50	1	0.55	0.50
unh1004	0.8	0.644	0.57	0.8	0.711	0.57	1	0.55	0.57
UNH185	1	0.55	0.50	1	0.55	0.50	1	0.55	0.50
GM538	1	0.55	0.50	1	0.55	0.50	1	0.55	0.500
GM531	0.8	0.66	0.54	0.80	0.60	0.54	0.80	0.533	0.54
Mean	0.8	0.517	0.47	0.77	0.51	0.47	0.88	0.536	0.47
SD	0.36	0.233	0.106	0.35	0.23	0.164	0.08	0.08	0.16

Table 5: Genetic differentiation (Fst) based on SSR (lower half) and RAPD markers (upper half) among the three *O.niloticus* families

	Family I	Family II	Family III
Family I		0.028	0.025
Family II	0.2613		0.029
Family III	0.4699	0.1028	

Only one locus (UNH136) was homozygous for all tested progenies within both families I and II, so inheritance tests could not be performed for them. On the other hand this locus was heterozygous in the third family (family III).

Out of the seven studied loci, only one locus (GM211) was informative in detecting two specific SSR markers (Table 2), to differentiate between family (I) and the other two families (II and III). The first one of the SSR markers was at fragment size of 398 bp which inherited from the L1 male parent. This marker was associated with the high growth rate characters in this economic fish and may be associated with the DNA regions affected this economic characters. On the other hand, the second SSR specific marker generated by the same primer at fragment size of 298 bp was found in the most individuals of families (II and III) since it was inherited from the low parents (L2 and L3). This marker could be considered as a negative marker for low growth rate characters in this fish.

The observed number of alleles (na), effective number of alleles (ne) and Shannon's information index (I) were calculated (Table 3) within each family and for all families for all primers.

Genetic Variation Within Each Family: The mean of Observed number of alleles (na), Effective number of alleles (ne), Nei's gene diversity (h), Shannon's Information index (I) and heterozygosity values were estimated and presented in Table 3. Family I had the highest na (2.7) and I (0.76) values. Family II had the highest ne value (2.04). Family III had the highest h (0.48) value (Table 3). The mean observed Heterozygosity values were 0.8, 0.77 and 0.88 in Families I, II and III, respectively (Table 4).

Genetic Variations among Families: Genetic differentiation values among the applied fish families were calculated. They were 0.26, 0.469 and 0.1028 between (I-II), (I-III) and (II-III) family pairs, respectively (Table 5).

DISCUSSION

According to PNGS [9], the genetic similarity within each of S, L1, L2 and L3 fish line was high (0.9 - 0.97). In addition, they found that using of (S) source as a female line gave a higher growth rate than the other studied sources. So (S) line was chosen to be the female parent in the present study. All applied fish families were produced from hybridization between the female line (S Line) with the different male lines (L1, L2 and L3). So the result divergent could be due to male components.

All studied families had a large number of allelic variants which mostly occurring in low frequencies. The same feature was observed by Romana-Eguia *et al.* [4] in GIFT project (genetically improved farmed tilapia).They hypothesized that, the SSR variability actually occurred in GIFT project strain was higher than that observed.

In the present study, SSR data showed that family I had the highest (na) value. The male parent (L1) of this family progeny was developed by PNGS project [9] from broad Egyptian *O.niloticus* genetic base, so it was expected that its progeny had a high (na) value.

Both L2 and L3 male parents were originated from private fish farm at Wadi EL-Natroon area in Egypt. They were produced through within family rotational mating selection (RMS) method [9]. This method (RMS) failed to maintain the stock's genetic variability. This could be due to unrestricted management in this farm. So, the mating of closely related individuals was not minimized. The homozygote excess [9] in these fish lines suggested that inbreeding was not effectively minimized. the comparison between L2 and L3 fish lines with FAC stock (Freshwater Aquaculture Center) showed that the RMS method helped to maintain the stock's genetic variability by minimizing the mating of closely related individuals [15].

In the present study, all SSR loci were polymorphic, so the primers of these loci are recommended to detect the heterozygosity and polymorphism in *O.niloticus* genome.

The present study showed that, GM211 locus was informative in detecting specific DNA marker at fragment size of 398bp to differentiate between family (I) and the other two families (II and III). This locus is located on chromosome 22 in *O.niloticus* [12]. This SSR marker which inherited from the L1 male parent was associated to the high growth rate characters in this fish. Both this SSR (GM211) and RAPD (generated by A1, C3 and C20 primers) markers may be associated with DNA regions which affect these economic characters.

In the present study, the mean of genetic differentiation (Fst) values were 0.268 and 0.149 based on RAPD and SSR analyses, respectively. So, both RAPD and SSR analyses were succeeded in detecting genetic divergence in the applied families. The Fst value is usually calculated for different genes and then averaged across all

loci and all groups. moreover genetic differentiation (Fst) is always positive [16]; it ranges between 0 (no subdivision) and 1 (extreme subdivision).On the other hand, there are minor differences among the average of Shannon's Information index (I) and gene diversity (h) values based on both of the applied markers (RAPD and SSR). These values were clearly high from zero and reflected the presence of some genetic variability levels within each one of the studied families. This is due to the using of different male parents (male component).

In conclusion, the *O.niloticus* growth rate characters required more molecular and quantitative studies to develop more DNA markers which associated with these economic characters. Genetic markers developed in this study will play a valuable role in maintaining and monitoring future husbandry practices undertaken during the culture of the studied fish families in our farms. In addition, these markers will be used as a marker-assisted selection (MAS) in the coming *O.niloticus* breeding programs. The success of this approach would depend on the extent of gene expression, genetic control of quantitative traits and the influence of culture environments.

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