E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(6): 395-401

www.fisheriesjournal.com
Received: 28-09-2017
Accepted: 29-10-2017

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Genetic improvement of farmed Nile tilapia (*Oreochromis niloticus*) through selective breeding in Egypt

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Abstract
Nile tilapia, *Oreochromis niloticus* is one of the most economically important African fish. The present study illustrates the current state of a selection program that mainly focuses on growth rate of farmed Nile Tilapia. Nile tilapia populations were collected from three geographically separated locations; Kafr El-Sheikh (K), Faiyum (F) and Serow (S). The selection based on fish age, condition factor and the genetic variation between populations; parents of two years, high Condition Factor (CF) and genetically distant parents were selected. Fish were fed on different nutrition regimes in different seasons. Simple Sequence repeats (SSR) technique was applied to assess the genetic variation between the studied populations. The molecular examination revealed that Kafr El-Sheikh (K) and Faiyum (F) populations are the most genetically distant populations with the lowest genetic similarity, 0.103. Therefore, two combinations of mating were done between (K) and (F), (F♀X K♂) and (F♂X K♀). Moreover, selected males and females from each population were also mated; (K♀ x K♂), (F♀ x F♂), (S♀ x S♂). Differences in growth rate among the produced strains were recorded during a period of five months revealing that (F♂ × k ♀) followed by (F♀× k ♂) & (F♀× F♂) strains have the highest significant values of total weight. Therefore, these strains will be considered as brood stocks for the coming selection. The work in this investigation will continue for at least another four years.

Keywords: Nile tilapia, genetic improvement, SSR, genetic diversity, GIFT, growth rate

1. Introduction
Nile tilapia, *Oreochromis niloticus* is a tropical food fish native to Africa and is considered as one of the most important fish species for freshwater aquaculture [1]. Generally, tilapia species have many attributes that make it suitable to be farmed and to be investigated for the application of genetics in aquaculture [2]. In addition to its general hardiness, they have a high tolerance to adverse environmental conditions & overcrowding, ability to withstand low oxygen and wide range of salinity concentrations and resistance to disease [3]. Tilapias are able to survive and grow on a wide range of natural and artificial feeds, convert food efficiently and grow relatively fast and accepted by a wide range of consumers [4, 5]. All these facts caused a rapid expansion of tilapia farming as well as the worldwide dispersal; however, Asian countries are considered the largest tilapia producers [6, 7]. In Africa, Egypt is the main producer of tilapia (produce about 990 thousand tons annually). However, the production of tilapia by the other African countries is very little [3]. Therefore, Tilapia is a target species for several genetic improvement programs [8, 9]. By time, many strains have been developed and improved through several selective breeding programs for Nile tilapia that have been established and maintained globally such as, GIFT (1993& 1998) [10, 11], GET-EXCEL (2004) [12], and GST (2004) [13]. Most of those selective breeding programs have been established in Asia. Genetic Improvement of Farmed Tilapia (GIFT) project began in 1988 which was funded by the United Nations Development Program (UNDP) and the Asian Development Bank (ADB) [14]. A total of eight African and Asian tilapia populations for the genetic improvement program, Egypt was a source for these collected populations (Manzallah, Abbassa, Ismailia) [14]. One of the most important GIFT stains developed in Asia was the Malaysian GIFT strain [15]. The origin of this strain was the Gift strain developed by Eknath *et al*. in 1993; Bentsen *et al*. in 1998; Eknath & Acosta 1998 [9, 10, 16] which had been disseminated to 11 countries in Asia [17]

and the development of the Malaysian GIFT strain lasted for seven years from 2002 to 2009. However, the trials for the genetic improvement of Nile tilapia in Africa were rare, despite the fact that Africa has the global wealth of tilapia genetic resources and a great natural prospective for aquaculture development especially Egypt [6].

The main objectives of the current project are to establish, produce, maintain and improve the GIFT strain of Nile tilapia in Egypt and to distribute the produced GIFT strain for local and global use. Furthermore, to establish and generalize a genetic improvement program of Nile Tilapia.

2 Materials and methods

2. 1 Sample collection, Transport and Adaptation

Nile tilapia populations were collected from three different locations in Egypt (Fig. 1); Kafr El-Sheikh (K), Faiyum (F), and Serow (S) during July (2015). These populations were known to have high growth rate and they were transferred to the Research Station, National Institute of Oceanography and Fisheries (NIOF) in El-kanater El-khayria.

Fig 1: Map showing locations of the collected samples during the present study

The collected populations had different environments, ages, and weights. Therefore, the parents were subjected to quarantine procedures three months in the El-Kanater El-khayria Research station for detecting genetic differences among these strains that are coming from different environments. During this period, fish broad stock were weighed every 4 weeks in order to adjust the daily feed rate, which was 3% of the average fish body weight in summer and 1% in winter. Water quality parameters were continuously measured according to the American Public Health Association [18]. Water temperature, dissolved oxygen, ammonia, and pH were monitored during the experimental period to maintain water quality at the optimum range for Nile tilapia. Water temperature was recorded daily in each pond using a mercury thermometer (ºC) suspended at 30 cm water depth, dissolved oxygen was measured by a digital oxygen meter (Yellow Spring Instrument Co., model 58, OH, USA); total ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, CO, USA) and pH values were determined by a digital pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Waltham, MA, USA)**.**

2.2 Selection parameters

At the end of March 2016, Total length (TL; cm), and total weight (TW; g) were recorded for the collected parents. The selection of fish broad stock for breeding was based on: (1) The condition factor (CF) given by the formula CF= W*100/ L [3, 19, 20] where, W is fish weight (gram) and L is fish total length (cm). (2) Age, which was estimated using fish scale and observed under binocular Optika microscope [21]. (3) Genetic distance between populations. Genetically distant populations were primarily determined. Then individuals having two years with highest condition factor values were selected.

2.3 Molecular examination

2.3.1 Caudal fin collection for DNA Extraction

A representative sample from each population (50 female and 50 males) underwent molecular examination to assess the genetic variations among populations. The caudal fin were collected, and preserved in 70% ethanol in -20 ˚C for further analysis. DNA was extracted according to Asahida *et al*., 1996 using: 700ml of TNES-Urea Buffer and 30 µl of proteinase K (10 mg/ ml) for tissue lysing, Phenol: Chloroform: Isoamy Alcohol (25:24:1) was used two times with an equal volume with repeated centrifugation for removing lipids and dyes from the DNA extract, finally, DNA was precipitated using two volumes of ice cold 100% EtOH, left overnight in 100% EtOH and washed in 70% ethanol. DNA pellets were resuspended in Tris EDTA (TE) buffer.

2. 3. 2 SSR-PCR analyses

Five SSR primers were used to assess the molecular differences according to Lee *et al*., 2005. Primer codes, their sequences and their amplification conditions are presented in Table 1. The primer UNH-130, was found to be associated with fish size [24], but, it was difficult at this stage to apply this marker because of the absence of parent history. So, it will be applied later in Marker Assisted Selection (MAS) in the coming steps.

Table 1: Primers codes, Sequences and Annealing conditions of SSR primers.

Primer code	Primer sequence	Annealing conditions
UNH123	Forward 5' CATCATCACAGACAGATTAGA 3' Reverse 5' GATTGAGATTTCATTCAAG 3'	65◦ C/ 45 sec.
UNH130	Forward 5'AGGAAGAATAGCATGTAGCAAGTA3' Reverse 5'GTGTGATAAATAAAGAGGCAGAAA3'	65◦ C/ 45 sec.
GM211	Forward 5' GCAAGTTGAGAGGCTACTGT 3′′ ′Reverse 5' AAACAACCCACAACCTTAGTT3	63◦/ 45 sec. C
GM538	Forward 5' CAGCATGTTGTCTGGATCTTG 3′ Reverse 5' TTTGTTGCTGTGGTCTGTTCTT 3	63◦ C/ 45 sec.
UNH106	Forward 5' CCTTCAGCATCCGTATAT 3' Reverse 5' GTCTCTTTCTCTCTGTCACAAG 3'	63◦ C/ 45 sec.

Polymerase chain reaction (PCR) was performed in a 25 µl volume using BIOLINE master mix (2X My Taq Red Mix). The PCR mixture contained 12.5 µl master mix, 1 µl DNA template (final concentration 20 mg) and 1 µl of each primer (final concentration 0.25 µM). PCR was performed on a BIO-RADPCR System (BIO-RAD, T100 Thermal Cycler, USA) with the thermal profile: an initial denaturation at 95°C for 3 min. Afterwards, 35 cycles were followed: denaturation step at 95°C for 1 min, annealing temperatures (as presented in Table 1.), 1 min Extension at 72°C, with a final extension of 7 min at 72°C. PCR products were loaded to 2.5% agarose gel contains 2 µl of Eth Br (100 mg/ml), and electrophoresed.

2.3.3 Molecular data analysis

Gel images which were revealed via SSRs were analyzed using free software, Totallab V1.2 (Nonlinear Dynamics, USA) and SPSS (ver. 10) software to determine molecular sizes of the amplified fragments, their frequencies through samples, and their polymorphism type either monomorphic or polymorphic. Data were recorded as presence (1) or absence (0) of bands from the gel photo. The (0, 1) data was then introduced to SPSS software package to infer similarities and genetic distance among the applied fish populations by the Unweighted Pair-Group Method of Analysis (UPGMA) cluster analysis. Similarity matrix was calculated depending on these primers software and a phylogenetic tree was constructed among the studied populations using SPSS (ver. 10) software.

2.4 Selective breeding combinations

Based on parent age, condition factor and the molecular variations among populations, 60 females and 20 males were selected. Parents' production for fry was recorded as a result of each mating; Growth rate of the produced fry was also recorded. Growth rate of all the produced strains were compared in order to determine future selection directions to produce the GIFT strain.

2.5 First generation nutrition

For each of the produced strains, the produced fry were weighed every four weeks in order to adjust the daily feed rate, which was 5% of the average fish body weight in summer and 1.5% in the winter. The daily ration of feed was divided at two equal amounts offered at two times daily. The fish diets were formulated to contain (30%, crude protein) and isocaloric (4500 Kcal gross energy /kg diet) in summer, (25%, crude protein) and isocaloric (4400 Kcal gross energy /kg diet) in winter. The Composition and proximate analysis of the fish diets are presented in Table 2.

Table 2: Composition and proximate analysis of the fish diets

Feed Ingredients	Experimental Diets	
Fish meal (59.9%)	12.00	9.00
Soybean meal (44%)	26.00	26.00
Corn gluten	00	12.50
Yellow corn	37.35	30.00
Wheat bran	20.00	17.80
Sun flower oil	2.00	2.00
Fish premix[1]	1.50	1.50
Di-Calcium Phosphate	1.00	1.00
Lysine	0.05	00
Methionine	0.10	0.20
Total	100	100
Chemical analysis of the experimental diets (on DM basis)		
Dry matter %	88.74	88.04
Crude protein %	25.18	30.03
Ether extract %	5.03	4.72
Crude fiber %	4.82	4.61
Ash %	6.64	6.04
NFE[2]	58.33	54.61
Gross energy (Kcal/ kg)[3]	4424.0	4510.93

1 Each kg of Fish premix contained: $MnSO_4$, 40 mg; MgO, 10 mg; K_2SO_4, 40mg; $ZnCO_3$, 60 mg; KI, 0.4 mg; $CuSO_4$,12 mg; ferric citrate, 250 mg; Na_2SeO_3, 0.24 mg; Co, 0.2 mg; retinol, 40,000 IU; cholecalciferol, 4,000 IU; α - tocopherolacetate,400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; nicotinic acid, 300 mg; folic acid 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg [2]. Nitrogen free extract (NFE) = 100 − (CP + EE + Ash) [3]. Gross Energy Calculated based on 5.65 Kcal/g proteins, 9.45 Kcal/g fat and 4 carbohydrates Kcal/g according to Jobling (1983).

2.6 Growth performance measurements

Live body weight (BW, g) and body length (BL, cm) of each produced strain were recorded every 4 weeks. At the end of the fish rearing period, fish were weighed and the growth performance parameters were calculated as follows:

Total weight gain (TWG, g/fish) = final weight – initial weight

Average daily gain (ADG, g/fish/d) = total weight gain (g) /period (day)

Specific growth rate (SGR) = LnW2 – Ln W1/T X 100 (where, Ln = the natural log, W2 = final weight at certain period (g), W1 = Initial, Weight at the same period (g), T = period of experiment (day)) Survival rate (SR, %) = No. of surviving fish/total No. of fish at the beginning X100

2.6.1 Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). It was performed with SPSS statistical

software (version 17.0, SPSS, Richmond, VA, USA). The data were subjected for test of homogeneity of variances and Duncan post-hoc test. Duncan's multiple range test (Duncan, 1955) was used to compare differences among treatment means and additive level means at 5% significance level (Data were considered significantly different when $P < 0.05$).

3 Results

3.1 Molecular analysis results

Five SSR primer pairs were used to characterize the three studied Nile tilapia populations, Kafr El-Sheikh (K), Faiyum (F) and Serow (S), and to assess the genetic variation between them. The molecular data revealed from these SSR markers showed that Kafr El-Sheikh (K) and Faiyum (F) are the most genetically distant populations with the lowest genetic similarity, 0.103; the genetic similarity values among the studied populations are presented in Table 3. The Unweighted Pair-Group Method of Analysis (UPGMA) cluster analysis of the similarity matrix based on SSR analysis is shown in Fig. 2. The studied populations were separated into two clusters, the first cluster includes Faiyum (F), and Serow (S) with a genetic similarity 0.350, whereas the Kafr El-Sheikh (K) is separates alone as an outlier group.

Table 3: Proximity matrix among the studied population, Kafr El-Sheikh (K), Faiyum (F), Serow (S) based on SSR results

Proximity Matrix			
Location	**Matrix File Input**		
	K	**F**	**S**
K	1.000	0.103	0.190
F	0.103	1.000	0.350
S	0.190	0.350	1.000

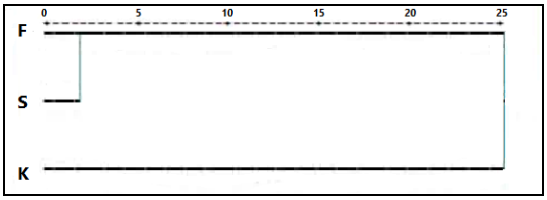

Fig 2: Dendrogram representing the phylogenetic relation among populations based on SSR analysis. K, Kafr El-Sheikh, F, Faiyum and S, Serow.

3.2 Growth rate follow up for parents

At the end of the adaptation period, it was noticed that the growth rate of El Faiyum (F) parents was the highest, followed by Kafr El-Sheikh (K) and the population having the lowest growth rate was Serow (S) population. Averages of BW and BL of Nile tilapia brood stock are presented in Table 4. Results revealed that, there was a significant difference in final live body weight and length for the different populations.

Table 4: The Initial and Final body weight, and body length for Nile tilapia brood stock

Population	Weight				Length			
	IBW (g)		FBW (g)		IBL (cm)		FBL (cm)	
	M	F	M	F	M	F	M	F
S × S	100.00^{b}	86.30^{b}	384.20^{b}	255.47^{b}	17.50	17.20	31.80^{b}	24.70^{b}
F × F	100.12^{b}	91.28	415.93^{a}	276.01	18.00	17.30	32.40^{a}	26.50
K × K	100.40^{a}	97.50^{a}	408.55^{a}	284.60^{a}	17.70	17.50	32.10^{b}	26.50^{a}
±SE	0.01	0.12	3.46	3.09	0.01	0.01	0.02	0.07

Means (± standard error), SE in the same raw with different superscripts are significantly different ($P< 0.05$) between each type. IBL: Initial Body Length, FBL: Final Body Length, IBW: Initial Body weight, FBW: Final Body Weight; M: Male; F: Female; K: Kafr El-Sheikh; F: Faiyum; S: Serow.

3.3 Selective Breeding direction

Both The growth rate measurements and the molecular variation analysis directed the mating to be between (K) and (F). Two combinations were done; (F♀ X K♂) and (K♀ X F♂). Moreover, selected males and females of each population were also mated (K♀ x K♂), (F♀ x F♂), (S♀ x S♂).

3.4 Offspring Growth Rate follow up

Quality parameters of rearing water for fish were recorded, water temperature ranged between (27 - 28ºC), pH values (7.5 and 9), dissolved oxygen ranged between (7 and 8.5 mg/l) and ammonia from (0.17 to 0.2 mg/l).

The Body Weight (BW) and Body length (BL) of the produced Nile tilapia strains are presented in Table 5. The results showed that, averages of final body weights and lengths had ranged between (104.78 – 126.00 g for males and 65.99 – 81.53 g for females, Fig. 4) and (17.3 – 18.1 cm for males and 14.8 – 15.5 cm for females) respectively. Significant differences in final BW and BL were recorded among the males and females of Nile tilapia strains.

Table 5: The Final body weight, Final body length of Nile tilapia produced strains

Population	Male		Female	
	FBW (g)	FBL (cm)	FBW (g)	FBL(cm)
S × S	17.3b	104.78c	14.8b	65.99d
F × F	18.0a	123.11a	15.2ab	76.46b
K × K	17.5ab	110.92b	15.0b	70.84c
F♀ × k ♂	17.7ab	119.85a	15.2ab	76.50b
F♂ × k ♀	18.1a	126.00a	15.5a	81.53a
±SE	0.02	2.67	0.02	1.95

Means (± standard error), SE in the same column with different superscripts are significantly different ($P< 0.05$) between each type. FBL: Final Body Length; FBW: Final Body Weight; M: Male; F: Female; K: Kafr El-Sheikh; F: Faiyum; S: Serow. Initial Body Length (IBL) was 1 cm for all; and the Initial Body Weight (IBW) was 0.5 g for all.

3.5 Strains comparisons based on growth parameters

Growth parameters and Survival rate (SR) of the produced strains were calculated. The analysis of variance for total weight gain showed that the highest significant (P<0.05) values of total weight gain was more pronounced in the fish group (F♂ × k ♀) followed by (F♀× k ♂) & (F♀× F♂) when compared with other produced strains (Table 6 and Fig. 5). The same trend was observed for specific growth rate (SGR) as presented in the same table. Concerning results of condition factor (CF), the lower significant (P<0.05) condition factor values were recorded for the fish group (S♀× S♂) compared to other strains. No significant differences (P<0.05) was observed for the survival rate (SR %) among these strains.

Fig 3: A harvest of the first generation of the producednile tilapia straius, Egypt

Table 6: The Nile tilapia offspring growth performance parameters

Populations	CF		WG (g)		ADG (g)		SGR (%/day)		SR (%)	
	M	F	M	F	M	F	M	F	M	F
S × S	2.02^{b}	2.04^{b}	104.28^{d}	65.49^{d}	0.74^{b}	0.47^{c}	3.82^{b}	3.49^{b}	100	99
F × F	2.11^{a}	2.18^{a}	122.61^{a}	75.96^{b}	0.88^{a}	0.54^{b}	3.93^{a}	3.59^{a}	100	98
K × K	2.07^{a}	2.10^{a}	110.42^{c}	70.34^{c}	0.79^{b}	0.50^{b}	3.86^{b}	3.54^{b}	99	100
F♀ × k ♂	2.16^{a}	2.18^{a}	119.35^{b}	76.00^{b}	0.85^{a}	0.54^{b}	3.91^{ab}	3.59^{a}	99	100
F♂ × k ♀	2.12^{a}	2.19^{a}	125.50^{a}	81.03^{a}	0.90^{a}	0.58^{a}	3.95^{a}	3.64^{a}	100	100
±SE	0.01	0.01	2.83	2.32	0.01	0.01	0.01	0.01	0.04	0.05

Means (± standard error, SE) in the same column with different superscripts are significantly different (P<0.05) between each type.CF: Condition Factor; WG: Weight Gain; SGR: Specific Growth Rate; Average Daily Gain; RGR: Relative Growth Rate; SR: Survival Rate; M: Male; F: Female; K: Kafr El-Sheikh; F: Faiyum; S: Serow.

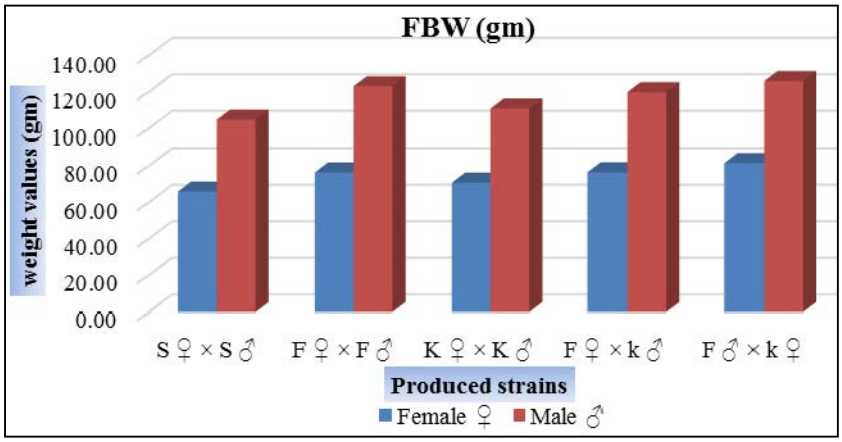

Fig 4: Comparison of The Final Body Weight (FBW) among different stains.

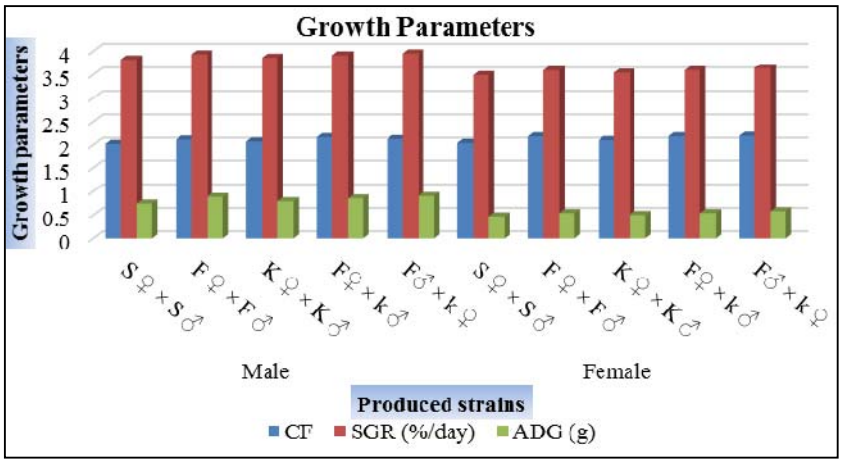

Fig 5: The offspring condition factor (CF), Specific growth rate (SGR), and Average daily gain (ADG) comparisons among the produced strains.

3.6 A future selection direction

Based on the current results, the future selective breeding will be focused on the Nile tilapia strains with the highest growth rate, which are (K♀ X F♂), (F♀ X K♂), and (F♀ X F♂). Strains having the lowest growth rate will be also considered, in order to estimate the differences in growth hormone between the large and small sized fish.

4 Discussion

The GIFT project is considered a superior example of a multidisciplinary and interactive research plan. This plan represents a selective breeding program which is supported by the studying of the genetic structure of parents and the produced strains. This could improve the growth performance and hence production of Nile tilapia strains in Egypt. All the tested water quality criteria were suitable for rearing Nile tilapia as cited by (Abdel-Hakim *et al*., 2002; Abdelhamid *et al*., 2002; Abdelhamid, 2009).

In this investigation, SSR markers were used as a tool for the assessment of the molecular variation among the collected parents. The used SSR markers were efficient in detecting the genetic polymorphism as reported by Saad *et al*., 2013. The analyses of the molecular data revealed from these SSR markers showed that Kafr El-Sheikh (K) and Faiyum (F) are the most genetically distant populations with the lowest genetic similarity. These results seem to be correlated with the geographical distance between the two locations; Faiyum is more geographically distant from Kafr El-Sheikh than Serow. This explanation is supported by Saad *et al*., 2009 [28].

The study of the genetic variation is a key step in any selection program, for the reason that, the genetic improvement depends mainly on maintaining the genetic variation between the mixed populations [29]. Therefore, the GIFT population must have additive genetic variance to enable further improvement with several generations of selection [29], Therefore, the introduction of new strains, for instance Nasser Lake population, may be needed.

The analysis of variance for total weight gain showed that the highest significant values of total weight gain was noticed in the fish strain (F♂×k♀) followed by F♀× k ♂ & F♀× F♂. This may be attributed to the high growth rate of their parents; Faiyum parents had the highest growth rate followed by Kafr El-Sheikh. In addition, the highest genetic variation was recorded between Kafr El-Sheikh (K) and Faiyum (F), the reason, that may causes the improved growth of the offspring.

The impacts of the GIFT project are mostly recorded as improved growth and production and in some cases; it can act to improve some economically important traits such as cold tolerance or salinity tolerance [2]. In addition, the evaluation of the GIFT strain is mostly recorded after three to five generations. For example, in Vietnam, faster growth and better tolerance at low temperatures were recorded for the GIFT strain after four generations of selection [3]; in this case, Growth has been improved by 20% [30]. In Fiji, The GIFT fish has been improved further through three generations of selection [31]. However, in the current state of this project, it is difficult to evaluate the produced Nile tilapia strains and to record the percentage of growth rate improvement, because only the first generation was produced. However, we can easily determine the directions of the coming selective breeding.

It will be possible to make an actual evaluation of the produced strains as they have the same ages, and exhibit the same environmental conditions. The evaluation of the produced strains should be focused on a group of parameters: (i) the strain superiority with regard to production traits, the long-term sustainability of that superiority which can be quantified by studying the build-up of inbreeding [32]. (ii) Maintaining the genetic variation within and among the produced strains [15]. (iii) The growth performance using the known growth parameters of each strain [15]. (iv) Fillet yield, chemical composition, flesh quality attributes, and fatty acid composition should be also evaluated for all strains [15].

5 Conclusions

The current study aims to genetically improve the growth performance of Nile tilapia through a selective breeding program. Both the molecular findings based on the SSR analysis and the growth rate of parents directed the mating to be between Kafr El-Sheikh (K) and Faiyum (F) populations as they had higher growth rate and higher genetic diversity. Nile tilapia strains resulted from the mating between genetically different populations, (K♀ X F♂), (F♀ X K♂), had higher growth rate compared to their counterparts produced by the mating between the females and males of the same population. Therefore, the brood stocks for the next selective breeding program were determined. This work will continue for at least another four years and it is expected, according to the current results, that there will be sustained gains of 10–15% per generation over more than six generations.

6 Acknowledgement

We delighted to accomplish the current research by the help of "Application of Breeding Methods for Production of Genetically Improved Farmed Nile Tilapia, *Oreochromis niloticus*" project, Funded by National Institute of Oceanography and Fisheries, Cairo, Egypt (NIOF), Ministry of High Education and Scientific research, which provided this work with the complete support and supplied it with the financial fund.

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