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Genetic Signatures of Some Egyptian *Clarias gariepinus* Populations

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Abstract: Molecular (Randomly Amplified Polymorphic DNA- Polymerase Chain Reaction; RAPD-PCR) and biochemical (Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis; SDS-PAGE) genetic markers of six *Clarias gariepinus* populations collected from different Egyptian locations (Zefta (El-Gharbia), El-Quanater, Mariut, El-Menia, El-Serw and El-Giza) were used to infer the genetic signature of each studied fish population. The protein banding patterns (SDS-PAGE) from skeletal muscle soluble proteins indicated that each population had a unique profile. The molecular data revealed from these genetic markers were used to reconstruct the dendrogram and calculate the genetic distance among the applied fish populations. The Unweighted Pair-Group Method of Analysis (UPGMA) cluster analysis of the similarity matrix based on RAPD analysis separated the studied populations into two clusters, the first formed by El-Menia, El-Serw and Mariut populations and the other by Zefta, El-Quanater and El-Giza populations. Some specific genetic markers were detected for each studied fish population. In conclusion, this investigation will be useful, especially in *Clarias gariepinus* breeding programs which use genetic markers as a marker assisted selection to improve the fish performance.

Key words: *Clarias gariepinus* · SDS-PAGE · RAPD-PCR · Genetic similarity · Dendrogram

INTRODUCTION

Catfish is a commercially important warm water fish which is distributed allover the world. The family of air-breathing or labyrinth catfishes (Clariidae) has over 100 representative species native to most of Africa and Southeastern Asia [1]. *Clarias gariepinus* (Burchell, 1822) is the native species of Africa. This species has drawn attention of aquaculturists because of its biological attributes that include faster growth rate, resistance to diseases and possibility of high stocking density. It has been introduced in several countries of Europe and Asia.

There is a number of different molecular tagging methods which have been designed to study fish populations. The use of biochemical methods such as isozymes and protein electrophoretic techniques for species identification has been widely applied in fish [2-6]. Also, the use of molecular methods such as RAPD technique [7, 8] AFLP technique [9-11] and microsatellite DNA [12, 13] has been widely applied in fish characterization.

The greatest advantages for RAPD technique are that it can potentially sample a large number of loci and that no prior DNA sequence information is needed to perform the assay[14].

The objective of this study was to detect the genetic signatures of six *Clarias gariepinus* populations collected from different Egyptian locations. This way will be of useful value, especially in *Clarias gariepinus* breeding programs which use genetic markers as a marker assisted selection to improve the fish performance.

MATERIAL AND METHODS

Sample Collection: Samples from*Clarias gariepinus* were collected during 2007. A total of 84 *Clarias gariepinus* individuals (14 individuals for each populations) was collected from six different Egyptian locations namely El-Gharbia-Zefta (Z), El-Quanater (Q), Mariut (R), El-Menia (N), El-Serw(S) and El-Giza (G), (Fig. 1).

Protein Analysis

Protein Extraction: A sample of 0.3 g of skeletal muscle from each individual was powdered using liquid nitrogen and extracted in appropriate volume of extraction buffer (0.85 % NaCl). In addition, 0.5 g of skeletal muscles was powdered using liquid nitrogen and extracted in appropriate volume of 70% ethanol, as described by Saad *et al.* [2] and Rashed *et al.* [15]. The powdered muscles were homogenized with extraction buffer for

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Fig. 1: Map of Egypt showing locations of *Clarias gariepinus* populations, studied locations are encircled

15 sec. The homogenized specimens were centrifuged at 12000 rpm /15 min /4°C. The supernatants were transferred to new Eppendorf tubes and kept in deep freezer until usage.

Protein Electrophoresis: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique was used to compare the studied *C. gariepinus* populations on the basis of their protein patterns. Saline and ethanol soluble protein fractions were performed exclusively on a vertical slab (19.8 x 26.8 x 0.3 cm) using the gel electrophoretic apparatus. The protein extract for all samples was applied to 15 % polyacrylamide gel. Gel preparation, electrophoresis conditions, staining and destaining procedures were done performed as outlined by Laemmli *et al.* [16].

RAPD Analysis: Approximately 1cm × 1cm of the caudal fin tissue was excised, preserved in70% ethanol and held at -4°C for subsequent DNA extraction. DNA extraction and purification were performed according to Hillis *et al.* [17]. A gene pool of the individuals representing each location was constituted. Ten primers (Operon technologies, Inc.; Alameda, California, EUA) were initially screened for consistently reproducible and scoreable amplified bands (Table 1). The primer codes were OPA12, OPB18, OPC01, OPC04, OPC02, OPC05, OPC9, OPC11, OPC12 and OPC19.

PCR mixture was prepared according to the instructions provided with Taq polymerase; catalog # M8301) purchased from Progmega Co. The reaction conditions involved initial denaturation of DNA for 4 minutes at 94°C, 30 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 37°C, 45 sec extension at 72°C and one 5 min cycle at72°C for final extension. The PCR products were separated on 1.5% agarose gels stained with ethedium bromide, run in 1X TBE buffer at a constant voltage of 80v.

Data Analysis: All gels were analyzed using totallab (version 2.01) and the SPSS ver.10 soft ware. Data were recorded as presence (1) or absence (0) of band products from the gel photographs. This data was then introduced to SPSS ver.10 soft ware package in order to infer similarities and genetic distances for interpopulation's relationships and also to calculate the similarity values within each applied fish population in addition to calculate the genetic distance among them.

Dendrogram Construction: The data generated from molecular markers was introduced to SPSS package program according to binary values (1, 0). The output results involved both different hierarchical pair-wise distance (UPGMA) and constructed dendrogram.

RESULTS

Protein Analysis: Concerning the protein electrophoresis results, 14 individuals from each location were sampled and their protein extract were applied to 15 % polyacrylamide gel. The protein banding patterns obtained from (SDS-PAGE) indicated that each population had a unique banding pattern. The total number of recorded bands, the range of molecular weight, the average of similarity values and the average of band frequency within each population are presented in Table (1).

Regarding the SDS-PAGE for saline soluble muscle proteins, a number of population specific protein markers were recorded, these markers are four specific protein bands, one for S population with a molecular weight of 33 KDa and three specific protein bandsfor Z population with molecular weights of 7, 8. and 9 KDa.

Regarding the SDS-PAGE for ethanol soluble muscle proteins, three specific protein markers were recorded. These markers were one protein bands unique for R population with molecular weights of 177 KDa and two protein bands unique for Z population with molecular weights of 14 and 13 KDa.

RAPD Analysis: RAPD comparative analysis was carried out using ten decamer primers to observe the degree of genetic similarity among the six studied populations (Z, Q, R, N, S and G).

Table 1: The total number of recorded bands, range of molecular weight, average of similarity values and the average of band frequency within each studied fish population Avr.: Average, BN: Band number, R.MW: molecular weight range, SE: Standard error, BF: Band Frequency

	Saline-SDS PAGE					Ethanol-SDS PAGE				
Population	Total BN	R.MW (KDa)	Avr. Similarity values	SE	X BF	Total BN	R.MW (KDa)	Avr. Similarity values	SE	Avr. BF
Z	36	165-7	0.975	0.0020	0.972	23	163-13	0.932	0.0067	0.58
Q	34	167-11	0.915	0.0020	0.836	23	157-30	0.911	0.0041	0.894
R	36	164-31	0.909	0.0107	0.877	20	177-28	0.907	0.0087	0.757
N	37	179-16	0.902	0.0040	0.788	31	154-20	0.935	0.0031	0.901
S	35	164-20	0.907	0.0060	0.816	29	168-20	0.909	0.0036	0.823
G	29	174-23	0.907	0.0115	0.652	26	161-26	0.908	0.0041	0.884

Table 2: Primer codes, sequences, total number of band detected and average of band frequencies generated by the ten RAPD primers

Primer code	Primer sequence	Total BN	Polymorphic BN	No. of Specific -DNA markers	Avr. BF
OPA12	3'-TCG GCG ATA G-5'	6	4 (66.7%)	1(G)	0.583
OPB18	3'-CCA CAG CAG T-5'	10	9 (90%)	1(Q)	0.533
OPC01	3'-TTC GAG CCA G-5'	8	4 (50%)	0	0.896
OPC02	3'-GTG AGG CGT C-5'	10	6 (60%)	1(S)	0.617
OPC04	3'-CCG CAT CTA C-5'	12	10 (83.3%)	1(G)	0.611
OPC05	3'-GAT GAC CGC C-5'	8	6 (75%)	1(S)	0.624
OPC9	3'-CTC ACC GTC C-5'	11	8 (72.7%)	1(S) and 1(G)	0.664
OPC11	3'-AAA GCT GCG G-5'	7	4 (57%)	1(M)	0.808
OPC12	3'-TGT CAT CCC C-5'	12	5 (41.7%)	1(G)	0.791
OPC19	3'-GTTGCCAGC C-5'	11	10 (90.9%)	1(R) and 1(G)	0.62

Table 3: The similarity values among the investigated six fish populations based on RAPD analysis

	Z	Q	R	N	S	G
Z						
Q	0.819					
R	0.701	0.774				
N	0.748	0.800	0.817			
S	0.694	0.750	0.712	0.823		
G	0.693	0.806	0.758	0.738	0.703	

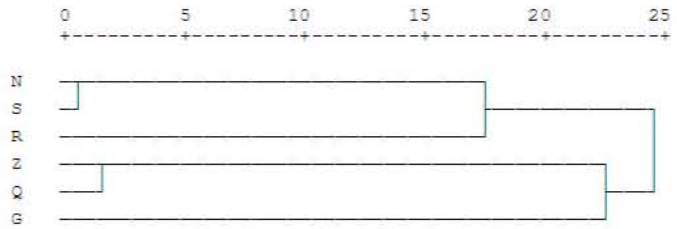

Fig. 2: Dendrogram representing the inferred phylogenetic relationships among applied fish population based on RAPD analysis

A total of 95amplified bands were detected of which 66 (69.5%) were polymorphic. The number and size of fragments were amplified by these primers varied from 6-12 bands and 148.5-1636 bp, whereas the average number of polymorphic bands varied from 4-10 bands. The highest number of amplified fragments (12 fragments) was produced by primers C4 and C12. The highest number of polymorphic fragments (10 fragments) was produced by primers C4 and C19.All the applied primers with exception of primer C1 generated population- specific RAPD markers (Table 2).

The UPGMA cluster analysis of the similarity matrix based on RAPD analysis separated the studied *C. gariepinus* populations into two clusters, the first formed by N, S and R populations while the other by Z, Q and G populations (Fig. 2) . In the first group, the dendrogram clearly showed that N, S was closely related with a similarity value of 0.823 (Table 3). Regarding to the second group, the dendrogram clearly showed that Z, Q were closely related with a similarity value of 0.819.

DISSCUSSION

It is important to study the aquatic organism's biodiversity, especially fish because they are the only major human food source that is primarily harvested from wild populations [18].

Many electrophoretic studies have been conducted to identify the differences among fish species over the entire world. In this regard, different protein sources were examined and included [19, 20]. The protein profiles for intra and inter-population identification was successfully applied. Serum and muscle proteins are commonly used to assess the polymorphism among fish species [19].

In the present study Protein electrophoresis SDS-PAGE were employed to study the genetic structure of six *C. gariepinus* populations. As postulated by Rashed *et al.*[19] Saad[20] and Smith [21].

Regarding the SDS-PAGE for saline soluble muscle proteins, the similarity values were 0.975, 0.915, 0.909, 0.902, 0.907 and 0.907 within Z, Q, R, N, S and G populations, respectively.

Regarding the SDS-PAGE for ethanol soluble muscle proteins, the similarity values were 0.932, 0.911, 0.907, 0.935, 0.909 and 0.908 within Z, Q, R, N, S and G populations, respectively.

The current results showed that, the average of similarity values within each studied fish population was high (based on muscle protein polymorphism). This observation was found in many fish studies such as in Tilapia (*O.niloticus*, *O. Aureus* and *Tilapia zillii*) as reported by Saad *et al.* [2] and Rashed *et al.* [15] and Catfish as reported by Rashed *et al.* [19].

Rashed *et al.* [19] studied some African catfish (*C. gariepinus*) populations collected from six different locations in Egypt, to estimate intra-specific variation. The intra-specific variations were low with exception of plasma profiles; it was higher than that obtained in the case of muscle profiles. They confirmed that, the muscle protein was considered as species-conservative that leads to the low intra-variations could be detected. In contrary, the blood plasma protein could be varied according to different entire conditions such as different physiology, sex-hormone, maturity stage, etc. These observations, in regard to muscle protein profiles, might be due to the stability of catfish during its evolutionary history. They used saline solution, NaCl (0.85%) as an extraction buffer for muscle soluble proteins. In the present study, two extraction buffers (0.85% NaCl and 0.70% ethanol) were used separately to get two muscle protein profiles. Using the two extraction buffers, the intra-specific variations were also low. So these results are in agreement with Rashed *et al.* [19] in this point.

Concerning the RAPD results, several authors have demonstrated that the RAPD method is powerful tool in the assessment of genetic markers which are capable of discriminating between species or subspecies in a wide range of organisms, including fishes [7, 8, 22, 23]. Almeida *et al.* [24] successfully used the RAPD technique to distinguish between two species (*Pimelodus cf. absconditus and Iheringichthys labrosus*) that presented very similar external morphology.

In the present study, the analysis of RAPD data succeeded in screening the variations among the applied *C. gariepinus* populations.

To detect the genetic polymorphism in different species or different populations of the same species using RAPD technique, some RAPD results follow the geographical distance; others not follow the geographical distance. This occured in different fishes and different locations. Such as that proved by Ambak *et al.* [25]and Bektas and Belduz [26].

In the present study, the UPGMA cluster analysis of the similarity values based on RAPD results separated the studied fish populations into two clusters, the first formed by N, S and R populations whereas, N, S were closely related with a similarity value of 0.823. This relation doesn't follow the geographical distance. This may be attributed to transportation and mixing processes that may dilute any differences in general phenotypic and

genotypic characters. This was agrees with Bektas and Belduz [26]. They used RAPD technique to investigate the DNA polymorphism among eight populations of whiting, *Merlangius merlangus euxinus* (family Gadidae) in the Black sea coast of Turkey using 11 arbitrary primers. Most primers generated very little differences despite the presence of oceanographic barriers for eggs and larvae of the eastern and western Black sea whiting . They attributed these results to the partial or complete transportation and mixing of eggs and larvae.

The second group (In the present study) formed by Z, Q and G populations. Where Z, Q were closely related with a similarity value of 0.819. This relation follows the geographical distance.

This explanation agrees with Ambak *et al.* [25]. They used RAPD technique to study the relation ships among four populations of Snakehead fish, Channa striate (Perils, Perak, Terengganu and Johore) (distributed in Malaysia). Eight arbitrary decamer primers were used and produced 42 polymorphic bands. the Dendrogram showed that genetic relationships follows the geographical distance.

In conclusion, this study revealed a relatively high level of genetic diversity which is required for populations to be more adaptive with the environmental changes. RAPD based dominant markers needs to be screened over more loci to obtain high accuracy of genetic variation estimates. Both RAPD and protein electrophoresis techniques are valuable techniques in investigating the genetic diversity in *C. gariepinus* populations. The genetic markers which detected in the present study will be useful, especially in fish breeding programs which use genetic markers as a marker assisted selection to improve the *C. gariepinus* performance.

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