Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 8, No. 4, p. 231-243, 2016
http://www.innspub.net

INNSPUB

RESEARCH PAPER

OPEN ACCESS

Genetic diversity in egyptian populations of *Tilapia* species using RAPD and SRAP markers

Gomaah El Fadly*, Ismael Khatab, Medhat Rehan, Alaa Kalboush

Department of Genetics, Kafrelsheikh University, Egypt

Article published on April28, 2016

Key words: Genetic diversity, Population differentiation, RAPD, SRAP, *Tilapia* species.

Abstract

This study aimed to estimate the genetic diversity among and within populations of three *Tilapia* species *e.g.,Oreochromis niloticus*, *Tilapia zilli* and *Oreochromis aurea* which were collected from different locations *i.e.,* Ryad, Motobs (Kafr El Sheikh Governorate), and Bahr El Baqar (Sharqia Governorate).Random amplified polymorphic DNA (RAPD) and sequence related amplified polymorphism (SRAP) were used in this study. Our results indicated that percentages of polymorphism were 58.41, 41.87 and 31.49% according to RAPD whereas 68.96, 33.33 and 17.64% terms of SRAP for *O. niloticus*, *T. zilli* and *O. aurea* respectively. The percentages of polymorphism using RAPD and SRAP within *O. niloticus* populations were the highest values . Population from Ryad was closer to population of Bahr El Baqar in *O. niloticus*, while population of *T. zilli* and *O. aurea* from Bahr El Baqar was more similar to population from Motobs. The outcome of this study considered as baseline of genetic diversity which will be useful in breeding program and conservation biology of *Tilapia* species and a baseline analysis of the population differentiation found among of *Tilapia* species in Egypt.

***CorrespondingAuthor:**Gomaah El Fadly✉gomaah@yahoo.com

Introduction

Tilapia species are the most species in fresh aquaculture in tropics and sub tropics areas. It belongs to the family cichlidae and the order perciformes. *Tilapia* are divided into three genera based on breeding habitats: *Oreochromis*, the maternal mother brooders; *Sarotherodon*, the biparental and paternal mouth brooders ; and *Tilapia* , the substrate spawners. *Tilapia* species are a good example for evolutionary scientists because they are fast of growing, divers in morphology, resistance to disease, tolerance to water with low quality and easy for breeding. They are a great source for protein in human diets **(**Saad *et al*., 2012**;** Abd el kader *et al.,* 2013, Abdel-Hamid *et al.,* 2014).

Many markers were used to study genetic diversity in *Tilapia* fish such as morphological marker (Samaradivakara *et al.,* 2012), biological markers, cytological markers (Sofy *et al.,* 2008) and molecular markers (Abdel-Hamid *et al.,* 2014). However, molecular markers are more favorable because it can detect polymorphism in DNA level so it used for identification of species and subspecies, detection of genetic variation within and between populations or species, and genetic mapping(Ali *et al.,* 2004; Chandra *et al.*,2010; Chauhan and Rajiv;2010; Li *et al.*,2014). These techniques are so useful in fish genetic improvement programmes for fish production with a good economically traits and adaption to environmentally changes (Rashed *et al.,* 2009). Many molecular genetic markers were applied for fisheries studies such as randomly amplified polymorphic DNA (RAPD) (Abd el kader *et al.,* 2013), restriction fragment length polymorphism (RFLP) (Abdul Rahim *et al.,* 2012), amplified fragment length polymorphism (AFLP) (Wang *et al.,* 2000), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR) (Saad *et al*., 2012) and sequence related amplified polymorphism (SRAP) (Zhang *et al.,* 2014).

RAPD marker amplifies DNA without previous information about genome of species and use universal primer which is commercially available (Ali *et al.,* 2004; Rashed *et al.,* 2011). It is dominant marker and inherited as mendelian marker where polymorphism is detected by presence and absence of bands. (Chauhan and Rajiv 2010).There are several studies for fish dealing with genetic diversity, and genetic improvement, (Carleton *et al*., 2002; Bhassu *et al.,* 2004; Romana–Eguía *et al.,*2004; Hassanien *et al.,* 2004).these studies were used successfully in estimation of genetic diversity for fish populations. Moreover (Soufy *et al.,* 2009) evaluate common patterns of genetic variations among three species of *Tilapia* using RAPD.

Sequence related amplified polymorphism (SRAP) was used to study genetic diversty, genetic mapping, population structure of living organisms such as *Brassica napus* (Sun *et al.,* 2007), Cucumber (Zhang *et al.,* 2010), and lotus (Deng *et al.,* 2013) without previous information about genome sequencing (Li *et al.,* 2011). It is better than SSR, ISSR, RAPD for studding genetic diversity (Chen *et al.,* 2013; Zhang *et al.,* 2014) this marker use combination of primers which amplify open reading frame (Li and Quiors, 2001). The used marker consist of 17-18 base contain 13 to 14 base (core sequence) out of them 10-11 base at the 5′ end called filler sequence which are no specific followed by the sequence CCGG in the forward primer (amplify exone region) and the sequence AATT in the reverse primer (amplify intron region) and follow core sequence three selective base at the 3′ end. The filler sequences differ from primer to other. The polymorphism among species and genotype is arising from variation in the length of introns, promoter and spacers (Li *et al.,* 2014). To the best of our knowledge, there is no report concerning the molecular variability of *Tilapia* fish using SRAP. Genetic diversity that assists breeding programs is fundamental and very necessary to avoid genetic variability lost and in consequence inbreeding issues. Therefore, the objective of the present investigation was to estimate the genetic diversity among and within three *Tilapia* species; *Oreochromis niloticus*, *Oreochromis aurea* and *Tilapia zilli* from different

Egyptian locations by using RAPD and SRAP markers which will be useful in genetic conservation of *Tilapia* species and genetic improvement program.

Materials and methods

Fish collection

Three *Tilapia* species, i.e., *O. niloticus*, *O. aurea* and *T.zilli* were used in this study. Each specie contains three populations were collected from three different locations. Two of these locations belonging to Kafr El-Sheikh Governorate (3748.12 km^2), *i.e.*, Ryad (locates at Middle of Kafr El-Sheikh Governorate) and Motobs which located at North-West of Kafr El-Sheikh Governorate and adjacent to Mediterranean Sea, Al-Borolos lake and Nile River. The third one was from Bahr El Baqar from El Sharqia Governorate. Ten individuals from each population were used except Bahr El Baqar population of *O. aurea* since five individuals were used according to sampling limitation.

Genomic DNA extraction

DNA was isolated from muscle tissue according to (Eshak *et al.*,2010), 100 mg muscle tissue were grounded in TNES-urea buffer (10mM Tris-Hcl, 125mM Nacl, 10mM EDTA 2Na, 1% SDS and 8 M Urea) and 20 mg/ml proteinase K were added, then the mixture was incubated at 37 ºC overnight. DNA was purified with phenol: chlorophorm: isoamyl alcohol (25:24:1) and chlorophorm: isoamyl alcohol (24:1) respectively. DNA was precipitated with sodium acetate (3M) and ice-cold absolute ethanol. DNA pellet washed in 70% ethanol and air- dried. The pellet resuspended in 100 µl TE and incubated at 65ºC for one hour for dissolving. The concentration and purity of DNA were determined by UV-spectrophotometry at 260 and 280 nm (Sambrook *et al,*1989).

RAPD and SRAP amplification

RAPD analysis was performed in 20 µl reaction volume containing 1 µl template DNA (40 ng), 1 µl primer (10µM) , 8 µl dds H_2O and 10 µl 2x PCR master mix solution (i-Taq™) using five randomly primers as shown in Table (1). RAPD PCR was performed at initial denaturation at 94 ºC for 2 min, 35 cycles of 94 ºC for 20 sec, 30 ºC for 20 sec, 72 ºC for 3 min, and final extension cycle at 72 ºC for 3 min and 4 ºC until used. SRAP amplification was carried out using six combinations of forward and reverse primer as shown in Table (1). PCR reaction carried in 10 µl about 1 µl template DNA (40ng), 0.5 µl forward primer (5 Pmol.), 0.5 µl reverse primer (5 Pmol.), 3 µl dds H_2O and 5 µl 2x PCR master mix. the reaction was performed at 94 ºC for 4 min as initial denaturation, 5 cycles each consist of 94 ºC for 1 min, 35 ºC for 1min, 72 ºC for 30 sec and 30 cycles each consist of 94 ºC for 1 min, 50 ºC for 1 min, 72 ºC for 30 sec. final extension performed at 72 ºC for 10 min. PCR products were analyzed electrophoretacally in 1.5% agarose gel in 1X TAE followed by staining with ethidium bromide and photographed by gel documentation (UVP PhotoDOC- imaging system UK).

Data analysis

Amplification profiles for the used studied genotypes as a result of RAPD and SRAP were compared with each other, where (1) means presence and (0) means absence of bands. The data were used to estimate genetic similarity. The electrophoresis patterns of the reproducible banding patterns of each primer which produced by RAPD and SRAP were chosen for analysis. Pairwise comparisons between individuals were used to calculate the Jaccard coefficient using PAST program. Cluster analysis was performed to produce a denderogram using unweighted pair-group method with arithmetical average (UPGMA).

Results and discussion

In present study, RAPD and SRAP markers were used to estimate genetic diversity within and among populations of *O. niloticus*, *O. aurea* and *T. zilli* from different locations. Out of five primers applied for RAPD, only three ones of it were pointed in the present study (GOM1-9, GOM2-9 and GOM4-9) according to their polymorphism exhibition. The same trend was applied for SRAP technique; since

three out of six combinations (Em4me4 with *O. niloticus*; Em4me3 with *T. zilli and* Em4me3 with *O. aurea*) were introduced in the present study according to its highest values of variations among the applied individuals. The obtained results of these two techniques presented in Figs 1 and 2.

RAPD analysis

The numbers of amplified bands were detected depending on the used primers and populations of each *Tilapia* species. The number of detected bands, polymorphic bands and percentage of polymorphism shown in Table (2). Five random primers generated total bands of 202, 203 and 181in populations of *O. niloticus*, *T.zilli* and *O. aurea* with average of 40.4, 40.6 and 36.2 bands /per primer respectively. Out of them, 118 polymorphic bands (58.41%) were recorded with an average of 23.6 bands/primer in *O. niloticus*. Meanwhile; 85 polymorphic bands (41.87%) were scored with an average of 17 bands/primer in *T.zilli*. Using O.*aurea* 57 polymorphic bands (31.49%) with an average 11.4 bands / primer were scored. Populations of *O. niloticus* scored 70, 77 and 55 total bands in Ryad, Bahr El Baqar and Motobs respectively, out of them 43, 49 and 26 were polymorphic bands with polymorphism of 61.42 , 63.63 and 47.27 %.

Table 1. RAPD and SRAP primer sequences used for RAPD and SRAP.

No.	Primer	Primer sequence (5 ` - 3 `)
RAPD primers		
1	GOM1-9	5`-CCCAAGGTCC- 3`
2	GOM2-9	5`-CATACCGTGG- 3`
3	GOM3-9	5`-AGCATGGCTC- 3`
4	GOM4-9	5`-GACCAATGCC- 3`
5	GOM7-9	5`-AGAGCCGTCA- 3`
SRAP primers		
Reverse primers		
1	em1	5`-GACTGCGTACGAATTAAT- 3`
2	em4	5`-GACTGCGTACGAATTTGA- 3`
Forward primers		
3	me2	5`-TGAGTCCAAACCGGAGC- 3`
4	me3	5`-TGAGTCCAAACCGGAAT- 3`
5	me4	5`-TGAGTCCAAACCGGACC- 3`

*Combination of SRAP primers, em1+me2, em1+me3, em1+me4, em4+me2, em4+me3 and em4+me4.

Populations of *T. zilli* gave 72, 74 and 57 scorable amplified bands in Ryad, Bahr El Baqar and Motobs respectively. Out of these amplified bands, 32, 33 and 20 were polymorphic bands with polymorphism of 44.44, 44.59 and 35.08 %.

Populations of *O. aurea* scored 65, 58 and 58 band in Ryad, Bahr El Baqar and motobs respectively. Out of them 30, 12 and 15 were polymorphic loci with polymorphism percentage of 46.15, 20.68 and 25.86. The highest polymorphic percentage (100%) was found in all populations of *O. niloticus* using primer GOM 2-9. However, the lowest polymorphic percentage (0.00%) was found in *O. aurea* in population Motobs using primer GOM 1-9and primer GOM 3-9. In total using overall primers, *O. niloticus* still gave the highest percentage of polymorphic with values 61.42, 63.63 and 47.27 compared with *O. aurea* which gave the lowest percentage of polymorphism 46.15, 20.68 and 25.86 for Ryad, Bahr El Baqarand and motobs respectively.

SRAP analysis

Six SRAP combinations were used for selected populations which exhibited highly RAPD variation to test the variability on those populations. As pointed in table (3), the six combinations scored 58, 87 and 68 bands in populations of *O. niloticus*, *T. zilli* and *O. aurea* with an average of 9.66, 14.5 and 11.33 fragment /primer pairs respectively. Total of 58 scorable amplified bands of which 40 were polymorphic band with an average of 6.66 polymorphic band/primer pairs. The percentage of polymorphism between the individuals within population of *O.niloticus* (Ryad, Bahr El Baqar and Motobs) were 50,85 and 72.22 % respectively.

Table 2. Total number of amplified bands, No of polymorphic bands and % of polymorphic bands using RAPD analysis in populations of three *Tilapia* species (*O. niloticus* , *T. zilli* and *O. aurea*) with five random primers (Gom 1-9, 2-9, 3-9, 4-9 and 7-9).

	O.niloticus			*T. zilli*			*O. aurea*			Primers
	R	B	M	R	B	M	R	B	M	
no .of bands	17	17	8	17	13	11	16	14	14	GOM
No of polymorphic bands	15	13	4	12	9	5	10	2	0	1-9
% polymorphic band	88.23	76.47	50	70.58	69.23	45.45	62.5	14.28	0	
no .of bands	15	16	14	13	16	9	13	12	12	GOM
No of polymorphic bands	15	16	14	9	12	5	10	3	10	2-9
% polymorphic band	100	100	100	69.23	75	55.55	76.92	25	83.33	
no .of bands	14	15	8	10	12	8	10	9	8	GOM
No of polymorphic bands	7	7	2	4	5	2	2	1	0	3-9
% polymorphic band	50	46.66	25	40	41.66	25	20	11.11	0	
no .of bands	9	12	10	17	17	13	11	10	13	GOM
No of polymorphic bands	3	8	3	5	3	4	4	4	2	4-9
% polymorphic band	33.33	66.66	30	29.41	17.64	30.76	36.36	40	15.38	
no .of bands	15	17	15	15	16	16	15	13	11	GOM
No of polymorphic bands	3	5	3	2	4	4	4	2	3	7-9
% polymorphic band	20	29.41	20	13.33	25	25	26.66	15.38	27.27	
Total no .of bands	70	77	55	72	74	57	65	58	58	
Total No of polymorphic bands	43	49	26	32	33	20	30	12	15	
% polymorphic band	61.42	63.63	47.27	44.44	44.59	35.08	46.15	20.68	25.86	
Percentage of polymorphism	58.41%			41.87%			31.49%			
Total no .of bands	202			203			181			

*R: Ryad B: Bahr El Baqar M: Motobs.

Regarding *T.zilli* populations; out of the 87 total amplified bands 29 were polymorphic with an average of 4.83 bands/ primer pairs. Percentages of polymorphism of populations of *T. zilli* were 35.71, 36.66 and 27.58 % in Ryad , Bahr El Baqarand Motobs respectively .

Populations of *O. aurea* produced 68 bands. Out of them 12 were polymorphic with an average of 2 polymorphic bands/per primer pairs. Percentages of polymorphism were 34.78, 9.09 and 8.69 % in Ryad, Bahr El Baqar and Motobs respectively.

Similar to application of RAPD, when we SRAP primers combinations applied, populations of *O. niloticus* gave the highest percentage of polymorphic using em4 + me2 primers. Meanwhile, the lowest percentage of polymorphic was found in *O. aurea* (0.00 %) in all populations with exception of Ryad and Motobs populations with Em1 and Me2 & em4+ me4 combinations. Out of overall SRAP primer combinations, *O. niloticus* still showed the highest polymorphic percentage with values 50, 85and 72.22 for Ryad, Bahr El Baqar and Motobs respectively. On contrary; *O. aurea* exhibited the lowest polymorphic

percentages. The obtained SRAP results confirmed those obtained out of RAPD technique application.

There were many informative bands which were found in more than individual based on RAPD (Table 4) and SRAP (Table 5).Primer GOM 1-9 gave the highest ones (nine bands). In contrary primer GOM 3-9 gave the lowest informative bands (only five bands). Moreover, some bands were more common and found in some individuals from the three populations of all applied species such as band with size 1050 bp using primer GOM1-9 and band size 400 bp using primer Gom4-4.

Table 3. Total number of bands, No. of polymorphic bands and percentage of polymorphic bandsusing SRAP analysis in populations of three *Tilapia* species (*O. niloticus*, *T. zilli* and *O. aurea*).

	O.niloticus			*T. zilli*			*O. aurea*			Primers
	R	B	M	R	B	M	R	B	M	
no .of bands	3	3	3	3	4	1	3	2	3	Em1
No of polymorphic bands	1	3	2	3	3	1	1	0	1	+
% polymorphic band	33.33	100	66.66	100	75	100	33.33	0	33.33	Me2
no .of bands	3	2	3	2	4	4	4	4	4	Em1
No of polymorphic bands	2	2	3	1	1	1	0	0	0	+
% polymorphic band	66.66	100	100	50	25	25	0	0	0	Me3
no .of bands	4	5	5	6	5	6	7	7	7	Em1
No of polymorphic bands	2	5	5	2	3	2	3	2	0	+
% polymorphic band	50	100	100	33.33	60	33.33	42.85	28.57	0	Me4
no .of bands	3	4	1	4	3	4	2	2	2	Em4
No of polymorphic bands	3	4	1	1	0	1	0	0	0	+
% polymorphic band	100	100	100	25	0	25	0	0	0	Me2
no .of bands	1	1	1	5	5	5	1	1	2	Em4
No of polymorphic bands	0	1	1	1	1	0	0	0	0	+
% polymorphic band	0	100	100	20	20	0	0	0	0	Me3
no .of bands	6	5	5	8	9	9	6	6	5	Em4
No of polymorphic bands	2	2	1	2	3	3	4	0	1	+
% polymorphic band	33.33	40	20	25	33.33	33.33	66.66	0	20	Me4
Total no .of bands	20	20	18	28	30	29	23	22	23	
Total no of polymorphic bands	10	17	13	10	11	8	8	2	2	
% polymorphic band	50	85	72.22	35.71	36.66	27.58	34.78	9.09	8.69	
Percentage of polymorphism	68.96			33.33			17.64			
Total no .of bands	58			87			68			

*R: Ryad B: Bahr El Baqar M: Motobs.

Table 4. Informative bands using different RAPD primers.

S.P	Band size	*O. niloticus*			*T. zilli*			*O. aurea*		
		R	B	M	R	B	M	R	B	M
GOM	1400	-	+	-	+	+	-	-	-	-
1-9	1350	+	+	-	+	-	-	-	-	-
	1300	+	+	-	-	-	-	-	-	-
	1150	-	-	-	-	-	-	+	+	-
	1100	+	+	-	-	-	-	+	-	+
	1050	+	+	-	+	-	-	-	+	+
	900	-	-	-	-	-	-	-	+	+
	500	+	+	-	-	-	-	-	-	-
	300	+	+	-	-	-	-	-	-	-
	1500	-	-	-	-	+	-	+	-	-
	1400	-	-	-	-	+	+	-	-	-
GOM	1350	-	-	-	+	-	+	-	+	-
2-9	1300	-	-	-	-	-	-	+	-	+
	1150	+	+	-	-	-	-	-	-	-
	1100	-	-	-	+	-	-	-	-	-
	1050	-	-	-	-	+	+	-	-	-
	900	+	+	-	-	-	-	+	-	+
GOM	1150	+	+	-	+	-	+	-	-	-
3-9	1100	-	-	-	-	+	+	-	-	-
	1050	+	+	-	-	-	-	-	-	-
	900	+	+	-	-	-	-	-	-	-
	700	+	+	-	-	-	-	-	-	-

GOM	1500	-	-	-	+	+	-	-	-	-
4-9	1250	+	+	-	-	-	-	-	-	-
	1050	-	+	+	+	+	-	-	-	-
	800	-	-	-	-	+	+	-	-	-
	700	-	+	+	-	-	-	-	-	-
	400	-	+	+	-	+	+	+	-	+
GOM	1300	+	+	-	-	-	-	-	-	-
5-9	1280	-	-	-	-	+	+	-	-	-
	1220	-	-	-	-	+	+	-	-	-
	1150	-	+	+	-	-	-	-	-	-
	1100	-	+	+	-	-	-	-	-	-
	900	-	-	-	-	-	-	+	+	-

*R: Ryad B: Bahr El Baqar M: Motobs.

According to the similarity matrix for *Tilapia* species from different locations (complete data not shown but percentages located above the figures.) based on RAPD and SRAP analyses, the results showed that in populations of O. *niloticus*, highest genetic similarity value was observed within populations of Motobs, according to RAPD, which showed a good harmony with the polymorphic percentage. Meanwhile, according to SRAP, Ryad populations scored high similarity. With Regarding to *T. zilli* Populations, the highest genetic similarity value was scored within Motobs according to RAPD and SRAP analyses. On the other hand, *O. aurea* populations exhibited the highest genetic similarity within Bahr El Bakar by RAPD while populations of Motobs showed high similarity by SRAP.

Table 5. Informative bands using different SRAP primers.

S.P	Band size	*O. niloticus*			*T. zilli*			*O. aurea*		
		R	B	M	R	B	M	R	B	M
Em1+ me2	500	-	-	-	-	-	-	+	-	+
	300	-	-	-	+	+	-	-	-	-
	200	-	-	-	+	+	-	-	-	-
Em1+ me3	550	-	-	-	-	+	+	-	-	-
	500	-	-	-	-	+	+	-	-	-
	350	+	-	+	-	-	-	-	-	-
Em1+ me4	200	-	+	+	+	-	+	-	-	-
	100	-	-	-	+	+	-	-	-	-
Em4+ me2	400	+	+	-	-	-	-	-	-	-
	300	-	+	-	+	-	+	-	-	-
	100	+	+	-	-	-	-	-	-	-
Em4 + me4	900	-	-	-	-	+	+	-	-	-
	600	+	-	-	-	-	-	+	-	-

The UPGMA tree dendrogram Figs (3 and 4) constructed to show phylogenetic relationship within populations of *O. niloticus*, *T. zilli* and *O. aurea* based on RAPD and SRAP analyses. Populations of Ryad and Bahr El Baqar were separated from Motobs populations in *O. niloticus*. Populations of Bahr El Baqar were closer to populations of Motobs in populations of *T. zilli* and *O. aurea*.

The numbers of molecular markers were varied in their banding pattern according to the species and populations. These DNA markers introduced a useful value, especially in fish breeding programs, which use genetic markers as marker-assisted selection to improve the fish performance (Rashed*et al.,* 2009).

The same idea was tested by Rashed *et al.,* (2011), they used RAPD marker to detect the genetic variations among *Tilapia* specie since the values of similarity among *Tilapia* species were high. The molecular genetic markers are widely used to identify lines or strains, define stock diversity, monitor inbreeding, and diagnose simply inherited traits and even to improve stocks.

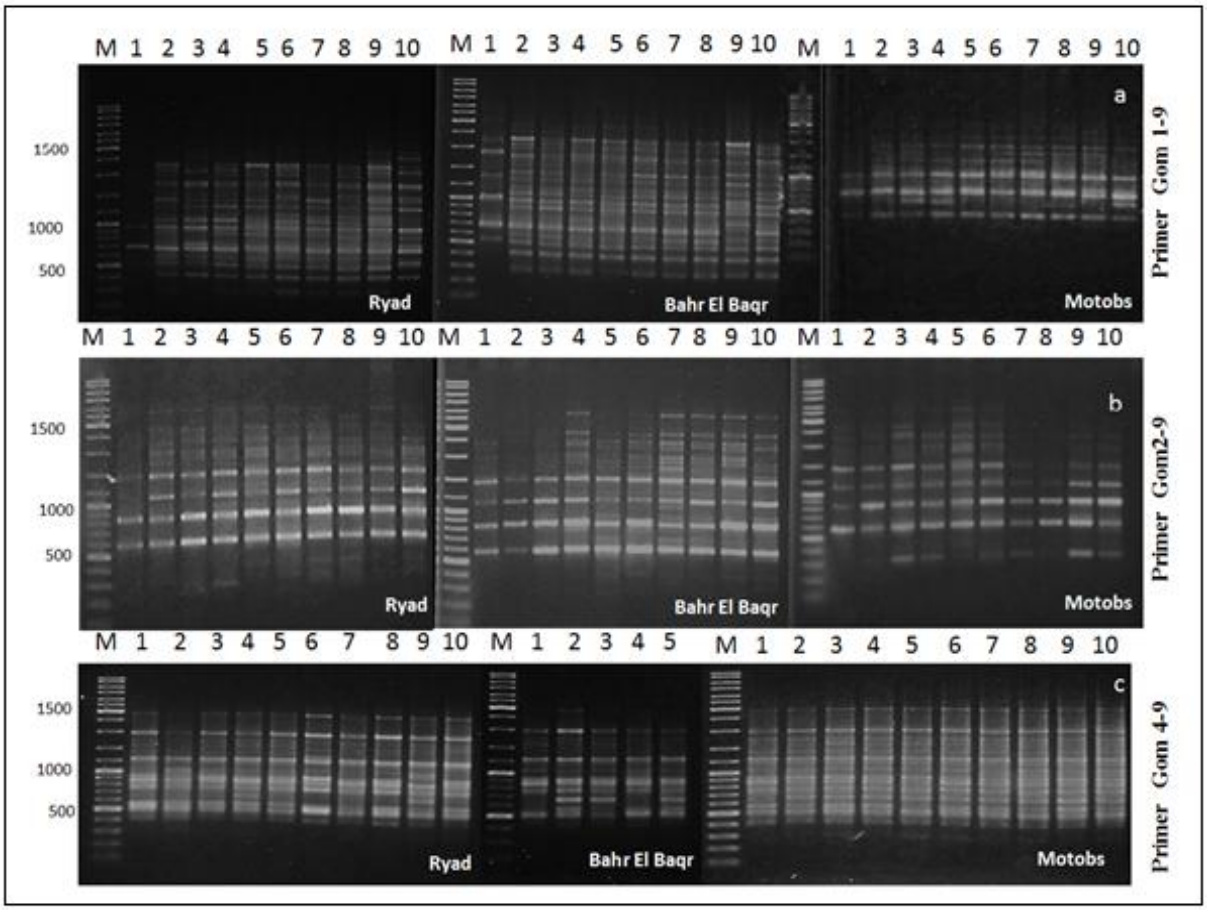

Fig. 1. Gel electrophoresis represents RAPD products of populations from *O. niloticus* (a) , *T. zilli* (b) and *O. aurea* (c) from different location (Ryad , Bahr El Baqar and Motobs) with primer Gom 1-9, Gom 2-9 and Gom 4-9.(M lane : 100bp Molecular Marker , lanes: 1→10 applied individual fish DNA template).

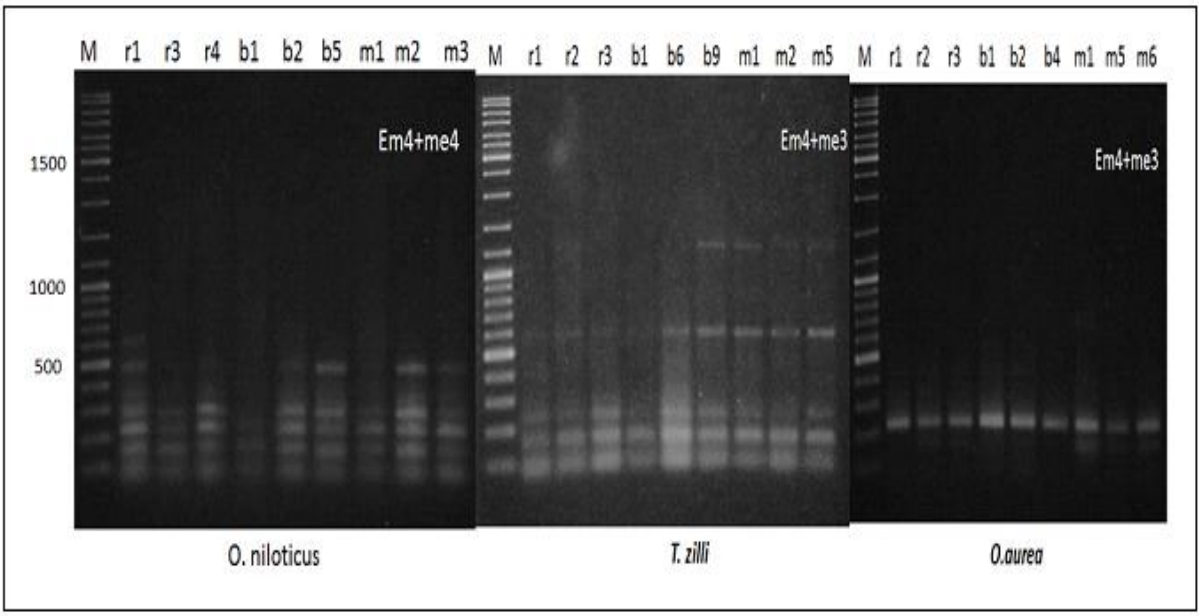

Fig. 2. Electrophoresis results of SRAP primers in population of *O. niloticus* (a), *T. zilli* (b) and *O. aurea* (c) from different location (Ryad , Bahr El Baqar and Motobs). (M lane: 100bp Molecular Marker, other 9 lanes presented ; r: Ryad b: Bahr El Baqar m: Motobas individuals DNA template.

The application of DNA-based genetic analysis as marker-assisted selection in fish research (such as *Tilapia*) and stock development and management is still not fully maximized (Kocher *et al.*,1998).However, Ahmed *et al,*(2004) and Hassanien *et al,*(2004) used random primers to assay RAPD polymorphisms among genera of *Tilapia* from Egypt, genetic diversity using RAPDs among different populations and high degree of polymorphism were detected. RAPD has also been used to estimate genetic diversity and variations required studying fish management and conservation practices, even with endangered species Shair *et al.,* (2011) and Mojekwu *et al.,* (2013). Pattern of species specific unique bands observed might be useful tools for molecular identification.

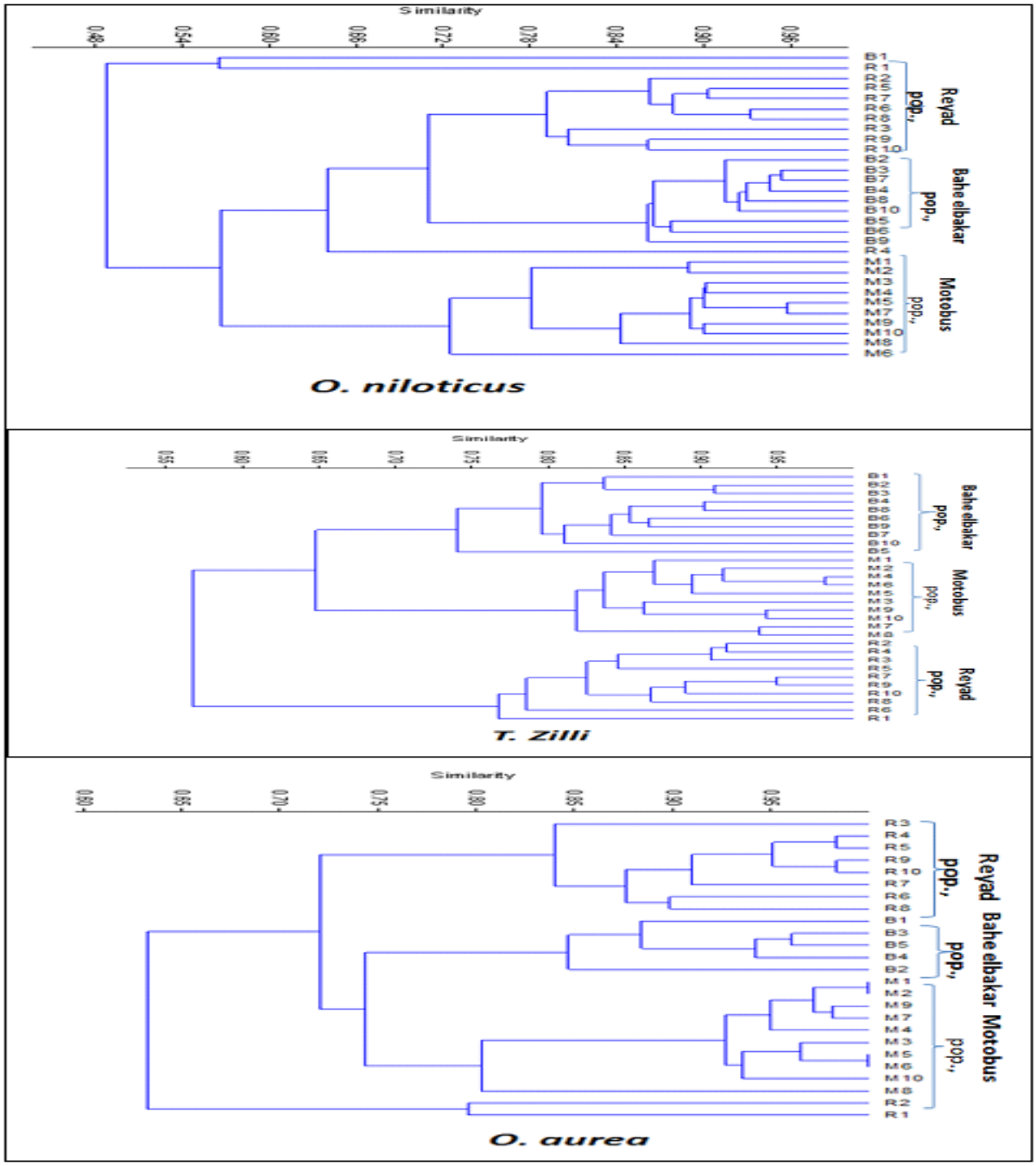

Fig. 3. Dendogram represent phylogenetic relationship within three populations of *O. niloticus*, *T. zilli* and *O. aurea* species based on RAPD data.

The diversity of DNA bands indicates the genetic distance between studied *Tilapia* species. However, the presence of common bands indicates evolutionary relationship. RAPD fingerprinting offers a quick and efficient method for generating a new series of DNA markers in fish (Bardakci2000; Islam and Alam 2004 &Abd El-Kader 2013). However, there was scare investigations on *Tilapia* based on SRAP (Ding *et al.*,2010) used SRAP and SCAR in molecular analysis of carp, recently (Zhang *et al.*,2014a) detected high variability among and between freshwater fish natural populations in China.

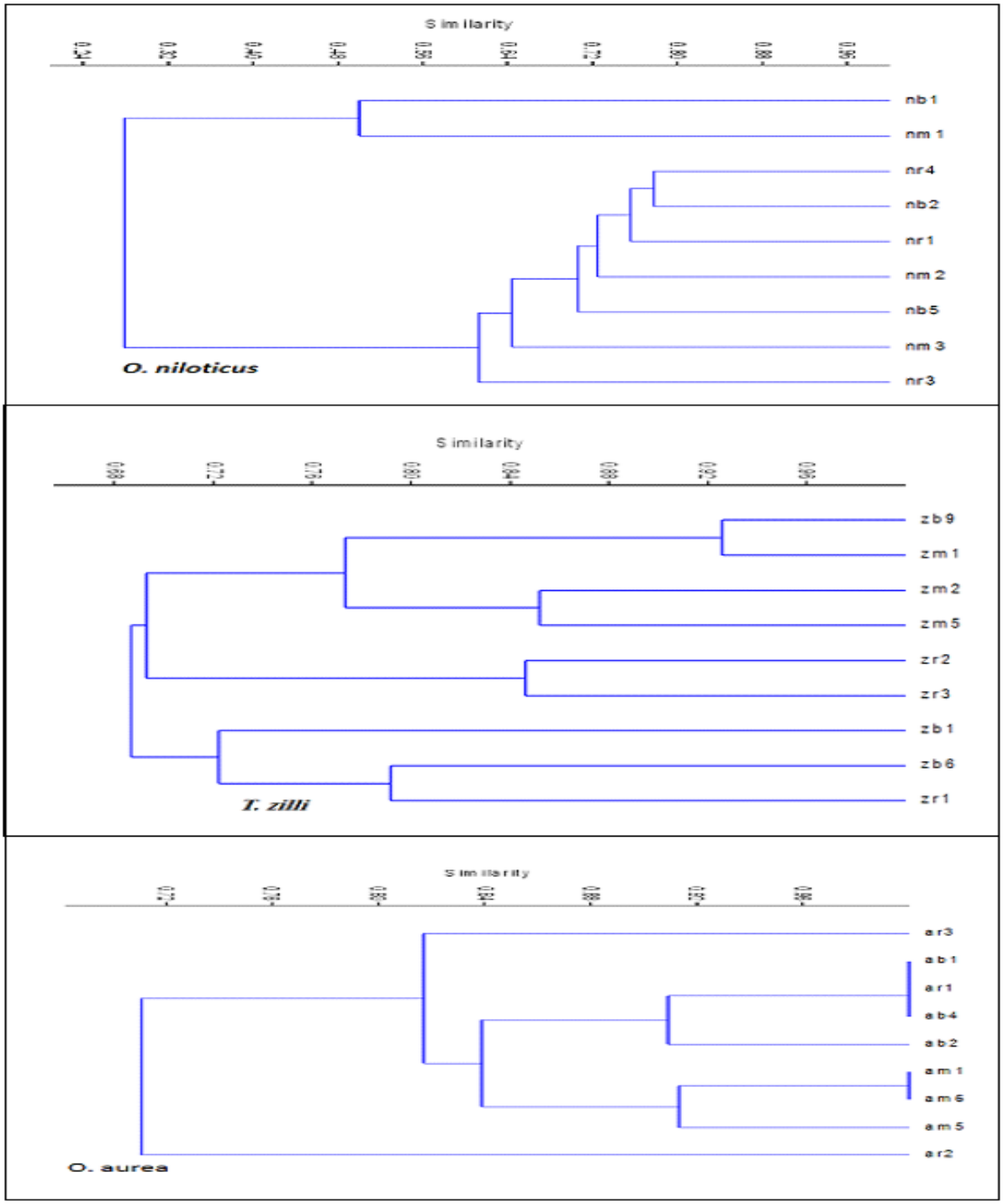

Fig. 4. Dendrogram represent phylogenetic relationship within three populations of *O. niloticus*, *T. zilli* , *O. aurea*from b = bahr el bakar population ; m = motobas population and r= reyad population based on SRAP data

Conclusion

The results obtained from the present study clearly showed that, the diversity of DNA banding patterns indicated the genetic distance between studied *Tilapia* species. However, the presence of common bands indicates evolutionary relationship.

Moreover; RAPD fingerprinting confirmed the previous knowledge about its application as a quick and efficient method for generating DNA markers in fish.Meanwhile, the SRAP technique exhibited a good harmony with the polymorphic statues within and between adopted species from the three different populations of the genera *Tilapia*.

It can be concluded also that, RAPD and SRAP proved to be a useful tool for estimating the genetic variability and degree of similarity among *Tilapia* species.Present study concluded also that the very high similarity between each population individuals leads to high probability of hybridization between them, but not among them. These findings are an indication for the distinct among populations from different regions and theses inbreed populations.

References

Abd el-kader H, Abd el hamid Z, Mahrous K. 2013. Genetic diversity among three species of *Tilapia* in Egypt detected by Random Amplified Polymorphic DNA marker . Journal of Applied Biological Science.**(7),** 57-64.

Abdel-hamid Z, Abdel-kader H, Aboelhassan D, Mahrous K. 2014. Genetic diversity in Egyptian *Tilapia* species using PCR-RFLP of D-loop Mitochondrial DNA gene. Research Journal of Pharmaceutical, Biological and Chemical Sciences**5,**469-475.

Abdul Rahim M, Ismail P, Alias R, Muhammad N, Jais A. 2012. PCR-RFLP analysis of mitochondrial DNA cytochrome b gene among Haruan (Channastriatus) in Malaysia. Elsevier**494,** 1–10.

Ahmed MMM, Ali BA, EL-Zaeem SY. 2004. Application of RAPD markers in fish: Part I- some genera (*Tilapia* ,*Sarotherodon* and *Oreochromis*) and species (*Oreochromisaureus* and *Oreochromisniloticus*) of *Tilapia*. International Journal of Biotechnology **6(1),** 86-93.

Ali B, Huang T, Qin D , Wang X. 2004.A review of random amplified polymorphic DNA (RAPD) markers in fish research. Reviews in Fish Biology and Fisheries. **14,**443-453.

Bardakci F. 2000. The Use of Random Amplified Polymorphic DNA (RAPD) Markers in Sex Discrimination in Nile *Tilapia* ,*Oreochromisniloticus* (Pisces: Cichlidae). Turkish Journal of Biology**24,**169–175.

Bhassu S, Yusoff K, Panandam M, Embong WK, Oyyan S, Tan SG. 2004. The genetic structure of *Oreochromis spp*. (*Tilapia*) populations in malaysia as revealed by microsatellite DNA analysis. Biochemical Genenetics**42,**217-229.

Carleton KL, Streelman JT, Lee BY, Garnhart N, Kidd M, Kocher TD. 2002. Rapid isolation of CA microsatellites from the *Tilapia* genome. Animal Genetics **33,** 140-144.

Chandra G, Saxena A, Barat A. 2010. Genetic diversity of two river in populations of Eutropiichthysvacha(Hamilton, 1822) using RAPD markers and implications for its conservation. Journal of Cell and Molecular Biology **8,** 77-85.

Chauhan1 T, Rajiv K. 2010.Molecular markers and their applications in fisheries and aquaculture. Advances in Bioscience and Biotechnology**1,** 281-291.

Chen S, Dai T, Chang Y, Wang S, Ou S, chuang W, Cheng C, Lin Y, Lin L , Ku H. 2013. Genetic diversity among *Ocimum* species based on ISSR, RAPD and SRAP markers. Australian Journal of Crop Science**7,** 1463-1471.

Deng L, Qin R, Gao J, Jia Y, Ren Y, GaoW, Lu L.2013. SRAP analysis of DNA base sequence changes in lotus mutants induced by Fe (+) implantation. Genetics and Molecular Research **12,**335-343.

Ding WD, Cao ZM, Cao LP. 2010.Molecular analysis of grass carp (*Ctenopharyngodon idella*) by SRAP and SCAR molecular markers. AquacultInt **18,** 575–587

Eshak M, Ebeed N, Booles H , Fahmy K. 2010. Genetic assessment of salinity tolerance in Red *Tilapia*.New York Science Journal**3,** 132-141.

Hassanien H, Elnady M, Obeida A, Itriby H. 2004. Genetic diversity of Nile *Tilapia* populations revealed by randomly amplified polymorphic DNA (RAPD). Aquaculture Research. **35,**587-593.

Islam MS, Alam MA. 2004.Randomly amplified polymorphic DNA analysis of four different populations of the Indian major carp, *Labeorohita* (Hamilton). Journal of Applied Ichthyology **20,**407-412.

Kocher T,LeeW,Sobolewska H, Penman D, Andrew B. 1998. A Genetic linkage map of a cichlid fish, the *Tilapia* (*Oreochromis niloticus*). Genetics, **148,** 1225-1232.

Li G, Quiros C.2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theoretical and Applied Genetics. **103,**455-461.

Li W, Zhang J, Mou Y, Geng J, McVetty P, Hu S, Li G. 2011. Integration of Solexa sequences on an ultradense genetic map in *Brassica rapa* L. Bio-Medical Central Genomics **12,**1-14

Li X , Li J, Zhao Z, Yang F, Fu Q, Liu H, Wang D, Yang Y, Wang R. 2014. Sequence-related amplified polymorphism (SRAP) for studying genetic diversity and population structure of plants and other living organisms: a protocol. The Journal of Animal & Plant Sciences **24,**1478-1486.

Mojekwu TO, Oguntade OR, Oketoki TO, Usman AB, Omidiji O. 2013. Molecular characterization of *Tilapia* in different water bodies using RAPD markers. African Journal of Applied Biotechnology Research**1,**1-12.

Rashed M, Saad Y, Atta A, Ahmed N. 2011.Reconstruction of phylogenetic relations among four *Tilapia* species. World Applied Sciences Journal**14,**456-462.

Rashed M, Saad y, EL – SeoudyA, Ibrahim M. 2009. Gene flow in some *Oreochromis niloticus* populations based on SSR linked markers to MHC loci class I. Boilogicai and Chemical Environmental Sciences. **4,** 319 – 331.

Romana-Eguia M, Ikedab M, Basiaoa Z, Taniguchib N.2004. Genetic diversity in farmed Asian Nile and red hybrid tilapia stocks evaluated from microsatellite and mitochondrial DNA. Aquaculture**236,**131–150.

Saad Y, Rashed M, Atta A, Ahmed N. 2012. Genetic diversity among some *Tilapia* species based on ISSR markers. Life Science Journal**(9),**4841-4846.

Samaradivakara S, Hirimuthugoda N, Gunawardana R, Illeperuma R, Fernandopulle N, Silva A, Alexander P. 2012. Morphological Variation of Four *Tilapia* Populations in Selected Reservoirs in Sri Lanka. Tropical Agricultural Research **23,**105-116

Sambrook L, Fritsch EF, Maniats T. 1989. Molecular Cloning: A laboratory manual ColdSpring Harbor Press. Cold Spring Harbor, N.Y.

Shair OHM, Al- Ssum RM, Bahkali AH. 2011. Genetic variation investigation of *Tilapia* grown

under Saudi Arabian controlled environment. American Journal of Biochemistry and Molecular Biology**(1),**89-94.

Sofy HI, Layla AM, Iman MKA. 2008.Karyotypic diversity of some *Tilapia* species. Nature and Science**(6),**19-27

Soufy H, Laila AM, Iman MKA. 2009. RAPD-PCR for DNA-Fingerprinting of Egyptian *Tilapia* .New York Science Journal**(2),**20- 25.

Sun Z, Wang Z,Tu J, Zhang J,Yu F,McVettyP, Li G. 2007. An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers.Theor. Applied Genetics **114,**1305-1317.

Wang Z, Jayasankar p, Khoo S, Nakamura K, Sumantadinata K, Carman O, Okamoto N. 2000. AFLP Fingerprinting Reveals Genetic Variabilityin Common Carp Stocks from Indonesia. Asian Fisheries Science**(13),**139-147.

Zhang GRW, Ran W, Gardner JPA, Wei KJ, Wang WM. 2014a. Genetic Diversity of and Differentiation among Five Populations of Blunt Snout Bream (*Megalobrama amblycephala*) Revealed by SRAP Markers: Implications for Conservation and Management. PLoS ONE**(9),** e108967.
http://dx.doi.org/10.1371/journal.pone.0108967

Zhang W, He H, Guan Y, Du H, Yuan L, Li Z, Yao D, Pan J, Cai R.2010. Identification and mapping of molecular markers linked to the tuberculate fruit gene in the cucumber (*Cucumis sativus L.***).** Theoretical and Applied Genetics **120,**645-654.

Zhang Y, Qin Y, Wang Z, Guo L, Chen X, Liang Z, Guo H. 2014b. DNA isolation and optimization of SRAP-PCR condition for endangered *Polyporus umbellatus*. Journal of Medicinal Plant Research, **(8),**133-137.