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Research Article

MOLECULAR CHARACTERIZATION OF SOME POPULAR FISH SPECIES IN SAUDI ARABIA

Ayman M. Sabry[1,2], Mohamed M. Hassan[1,3*], Alaa A. Mohamed[1,4] and Nabil S. Awad[5]

[1]Scientific Research Deanship, Taif University, kingdom of Saudi Arabia
[2]Cell Biology Department, National Research Centre, Dokki, Giza, Egypt
[3]Department of Genetics, Faculty of Agriculture, Minufiya University, Egypt.
[4]Department of Animal Reproduction and AI, Veterinary Research Division, National Research Center, Dokki, Giza, Egypt
[5]Department of Genetics, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt.
Corresponding author's email: khyate_99@yahoo.com

Abstract

The present work aims to molecularly characterize some popular Saudi fish species. Seven popular Saudi fish species namely, Morgan (Nemipteru sjaponicus), Mousa (Solea solea), Hamor (Greasy grouper), Shour (Lethrinus lentjan), Dennis (Caranxsex fasciatus), Harid (Scarus arabicus) and Black surgeon (Acanthurus gahhm) were characterized using six RAPD and three ISSR as well as Rep-PCR markers. These markers produced 187 distinct bands 89.4% were considered as polymorphic bands and 10.6% were considered as monomorphic bands. The RAPD OPA-06 primer revealed 100% polymorphism for 19 produced bands where band width ranged from 190 to 2100 bp. Likewise Rep-PCR ERIC1R primer exhibited 100% polymorphism for 18 bands produced bands where band width ranged from 50 to 1400bp. Data of the three types of genetic markers were combined for phylogenetic analysis. The resulted dendergram produced two large linages with around 59% genetic similarity. One linage only included Morgan (Nemipteru sjaponicus), and the other linage comprised the other six species. This result suggested that this six species were descended from Morgan fish. The second linage comprised two clades; the first clade contained four species (Mousa, Hamor, Shour, and Dennis), where the next clade included only Harid and Black surgeon. Generally genetic distance among native fishes was relatively low. The smallest genetic distance (0.512) was estimated between Shour fish and Black surgeon fish. To the best of our knowledge this work is breaking new ground in two directions, first, molecular characterization of Saudi fish, second employment Rep-PCR genetic marker for molecular characterization of fish species. This work could be considered as preliminary work towards an establishment of Saudi genetic conservation program.

Keywords: RAPD; ISSR; Rep-PCR markers; fish species; Saudi Arabia

Introduction

Generally, genetic markers provide the needed information for management of aquatic species such as fish in Saudi Arabia (Rashed *et al.*, 2008, Rashed *et al.*, 2009, Saad *et al.* 2011, Saad *et al.*, 2012), shrimp (Saad *et al.*, 2013). The advantage of Randomly Amplified Polymorphic DNA (RAPD) to generate molecular characterization is the production of molecular markers without any previous genomic information on the target species. RAPD assays have been used for estimating genetic diversity among different fishes (Saad *et al.*, 2013, Saad *et al.*, 2014). RAPD is a quick and effective method that can be applied to generate genotype with specific banding patterns (Hassan *et al.*, 2014; Ahmed *et al.*, 2014). RAPD was used for the analysis of genetic diversity in Saudi Arabia fish species, which had a significant impact on the fish genetic resources in Saudi Arabia (Rashed *et al.*, 2009, Saad *et al.* 2011).

Genetic divergence has been fully examined by Inter Simple Sequence Repeat (ISSR). The ISSR marker technique involves polymerase chain reaction (PCR) amplification of DNA using a single primer composed of a microsatellite sequence, the ISSR has mild technical difficulty, good reproducibility and reasonable cost, permitting its use for genetic studies of population (Chen *et.al,* 2005 and Li and Xia, 2005). ISSR was reported as more preferable tool than other markers for studying genetic divergence between quite near individuals (Fang *et al.*, 1997; Ullah *et al.*, 2015). Many studies showed that this approach could be used as a useful tool for the genetic diversity monitoring in different populations of animals (AhaniAzari *et al.*, 2007; Kol and Lazebny, 2006; Sabir *et. al* 2013 and Ahmed and Rezk, 2015). Latterly, ISSR marker tool has been applied to determine genetic variety and DNA polymorphism of some molluscs. Varela *et al.* (2007) assessed the genetic differentiation among *Mytilusedulis* mussels' complex collected from six sampling localities distributed along the European Atlantic coast by microsatellite markers. Dong *et al.* (2011) evaluated the genetic divergence of apple snail populations using ISSR analysis in China. However, ISSR assay was not applied

before to evaluate the genetic diversity among *Nemipteru sjaponicus*, *olea solea*, *Lethrinus lentjan* and *Caranxsex fasciatus* species, other molecular techniques were used by numerous researchers to study genetic diversity among different populations. Analysis methods such as Dice and simple match coefficients are commonly employed in the analyses of similarity and/or dissimilarity values among individuals in the absence of knowledge of ancestry of all individuals of species and sub species such as in Tilapia species (Rashed *et al*, 2011). The main objective is to molecularly characterize some popular Saudi fish species using different genetic markers RPDD, ISSR and Rep-PCR. Determining true genetic dissimilarity between individuals is a decisive point for clustering and analyzing diversity within and among aquatic species, because different dissimilarity indices may yield conflicting outcomes.

Materials and Methods

Sample collection

The sample collection and laboratory work of this study was conducted from March 2014 to May 2014. The fish samples of this experiment was conducted to study the efficiency of RAPD and ISSR markers for generating polymorphism in different fish species, Morgan fish (*Nemipteru sjaponicus*), Mousa fish (*Solea solea*), Hamor fish (Greasy grouper), Shour fish (*Lethrinus lentjan*), Dennis fish (*Caranxsex fasciatus*), Harid (*Scarus arabicus*) and Black surgeon fish (*Acanthurus gahhm*). Species individuals were collected based on their morphological characterization from Jeddah, KSA fish Market (Fig. 1). Three fish individuals were sampled from each collected fish species. From each specimen, approximately 1 x 1 cm of caudal fin tissue was excised, placed in a 70 % ethanol and held at 4°C for subsequent DNA extraction. The laboratory work was performed in the Biotechnology and Genetic Engineering Unit and Scientific Research Deanship, Taif University, KSA.

DNA extraction

Genomic DNA extraction and purification were performed according to (Hills *et al.*, 1996) and DNA Qiagen Kit (DNeasy Blood & Tissue), following the instructions of the manufacturer. DNA quality was checked by electrophoresis in a minigel and quantified using a spectrophotometer (Spectronic Genesys, Thermo Electron Corporation).

RAPD analysis

For RAPD analysis, seven 10-mer random primers were used (supplied by Amersham Pharmacia Biotech. NJ. USA.). Names and sequences of the primers are illustrated in Table 1. Following the experiments for optimization of component concentrations, PCR amplification of random primers were carried out according to Williams *et al.* (1990) and Saad *et al.*, (2012). in 25 µl volume containing 1µl (20 ng) of genomic DNA, 12.5µl of Go Taq® Green Master Mix, Promega, USA. 1µl of primer (20 p.mol), deionized distilled water (up to a total volume of 25 µl). For DNA amplification, the C1000TM Thermo Cycler Bio-Rad, Germany, was programmed under the conditions involving denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 30 Sec, primer annealing at 35°C for 1.5 min and primer extension at 72°C for 2.5 min; final extension step at 72°C for 7 min. Amplified DNA products were analysed by electrophoresis in 1.5% agarose gel run in TBE. The gels were stained with ethidium bromide (5 µg ml^{-1}). 100 pb. DNA Ladder RTU, (Gene Direx®) was used as a standard. DNA was visualized by UV illumination and then photographed by a Bio-Rad Gel Doc 2000 device.

Table 1: Names and sequences of the random primers used in this study.

Primer	5′→3′ sequence
OP-A2	TGCCGAGCTG
OP-A6	GGTCCCTGAC
OP-A8	GTGACGTAGG
OP-A9	GGG TAA CGC C
OP-A10	GTGATCGCAG
OP-B6	TGCTCTGCCC
ISSR-2	GAGAGAGAGAGAGAGAA
ISSR-7	CGAGAGAGAGAGAGAG
ISSR-10	CAGCAGCAGCAGCAGCAGCA
GTG-5	GTGGTGGTGGTGGTG
REP1R-I-F	ICG ICG ICA TCI GGC
REP2R-I-R	ICG ICT TAT CIG GCC TAC
ERIC1R-F	ATGTAAGCTCCTGGGGATTCAC
ERIC1R-R	AAGTAAGTGACTGGGGTGAGCG

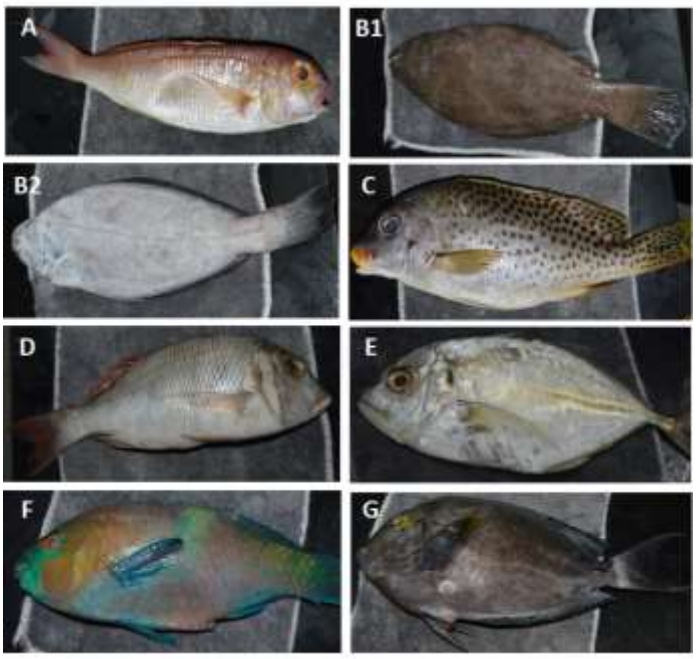

Fig. 1: Morphological identification of seven Saudi Arabia fish species, A= Morgan fish (Nemipteru sjaponicus), B1 and B2= the two faces of Mousa fish (Solea solea), C= Hamor fish (Greasy grouper), D= Shour fish (Lethrinus lentjan), E= Dennis fish (Caranxsex fasciatus), F= Harid (Scarus arabicus) and G= Black surgeon fish (Acanthurus gahhm).

ISSR and Rep-PCR analysis

For ISSR and Rep-PCR analysis, Go Taq® Green Master Mix, Promega, USA was used. The final total volume of each reaction was 25 µl; contained 0.625 units of Taq DNA polymerase, 2 mM MgCl2 and 0.2 mM of each dNTPs. 50 ng of DNA and 1000 p.mol of the primer were added to the reaction. PCR amplification of ISSR primers were carried out according to Fahmi and Al-Otaibi (2011). The thermocycler was programmed by an initial standard denaturation cycle at 94°C for 5 min. The following 40 cycles were composed of: denaturation step at 94°C for 30 sec, annealing step was programmed at different temperatures according to the primer used (40 to 58 °C) for 1.5 min and elongation step at 72°C for 2.5 min. The final cycle was polymerization cycle performed at 72°C for 7 min. The PCR products of each reaction were analyzed by electrophoretic separation in 1.5% agarose gel. 100 pb. DNA Ladder RTU, (Gene Direx®) was added on one side of the gel to determine the DNA patterns. Gel was stained by ethidium bromide (0.5 mg/ml). After electrophoresis, the ISSR patterns of the PCR products were visualized by UV illumination and then photographed by a Bio-Rad Gel Doc 2000 device.

Data analysis

The amplification products of RAPD-PCR were scored for the presence "1" or absence "0" and missing data as "9". The genetic associations between isolates were evaluated by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the proportion of shared bands produced by the primers. The dissimilarity matrix was generated using Neighbour joined method and hence dendrogram was reconstructed. The computations were performed using the program NTSYS-PC version 2.01 (Rohlf, 2000). The Jaccard′s similarity matrix was subjected to principal component analysis.

Results

For the three genetic markers (RAPD, ISSR and Rep-PCR) combined the total number of bands were 187 of which 167 bands were polymorphic (Table 2). Details of PCR amplification results for each of the three genetic markers follows:

PCR amplification of RAPD markers for seven Saudi fish species.

Genomic diversity of seven Saudi fish species was investigated by RAPD markers. Results of RAPD-PCR are illustrated in Table 2 and Fig. 2 and 3. RAPD-PCR reactions were performed with twenty samples and six different 10-mer primers. RAPD-PCR markers yielded 98 distinct bands of which 91 bands were polymorphic (92.8%) and only 7 bands were monomorphic (7.2%).

Number of bands for individual RAPD primers varied from 13 bands for OPA-02 to 19 bands for OPA-06. The highest polymorphism was recorded for OPA-06, where all the produced 19 bands were polymorphic. The band size of OPA-06 ranged from 190 bp-2100 bp. The lowest polymorphism (84.6%) was found for OPA -02.

Table 2: Polymorphic bands of each genetic primers and percentage of polymorphism in twenty one individual freshwater fish samples**.**

Primers	Total Bands	No. of Monomorphic Bands	No. Polymorphic Bands	% Monomorphic bands	% Polymorphic bands
OP-A2	13	2	11	15.4	84.6
OP-A6	19	0	19	00.0	100
OP-A8	17	2	15	11.7	88.3
OP-A9	16	0	16	00.0	100
OP-A10	18	0	18	00.0	100
OP-B6	15	3	12	20.0	80.0
ISSR-2	18	4	14	22.2	77.8
ISSR-7	16	3	13	18.7	81.3
ISSR-10	13	2	11	15.4	84.6
ERIC1R	18	0	18	00.0	100
REP1R-I	12	1	11	08.3	91.7
GTG-5	12	3	9	25.0	75.0
Total	187	20	167		

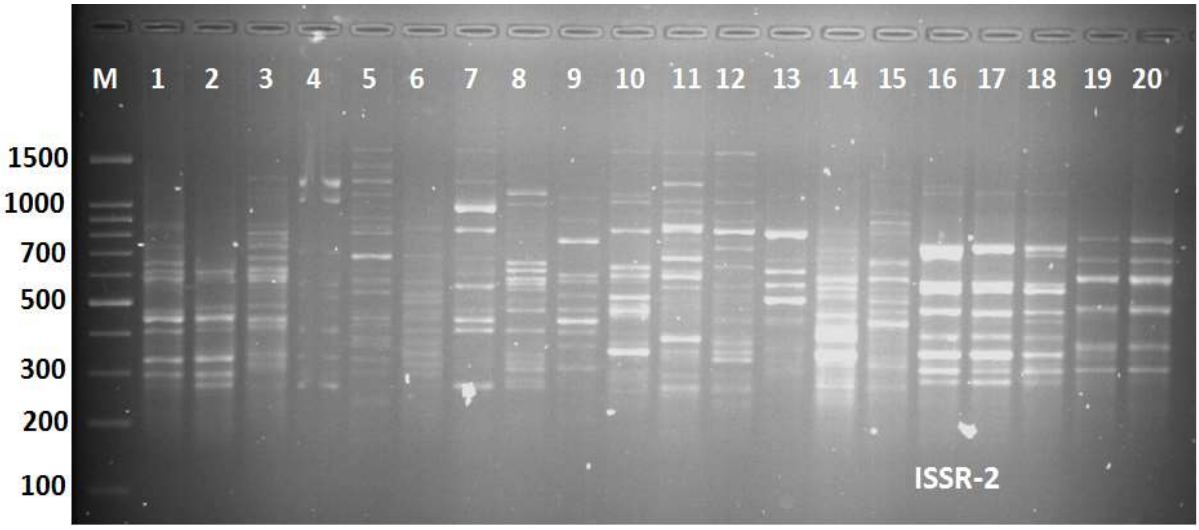

Fig. 2: ISSR profile of individual samples of seven fish species generated by primer ISSR-2. Whereas, 1-3= Morgan fish, 4-6 Mousa fish, 7-9= Hamor fish, 10-12= Shour fish, 13-15= Dennis fish, 16-18= Harid and 19-20= Black surgeon fish.

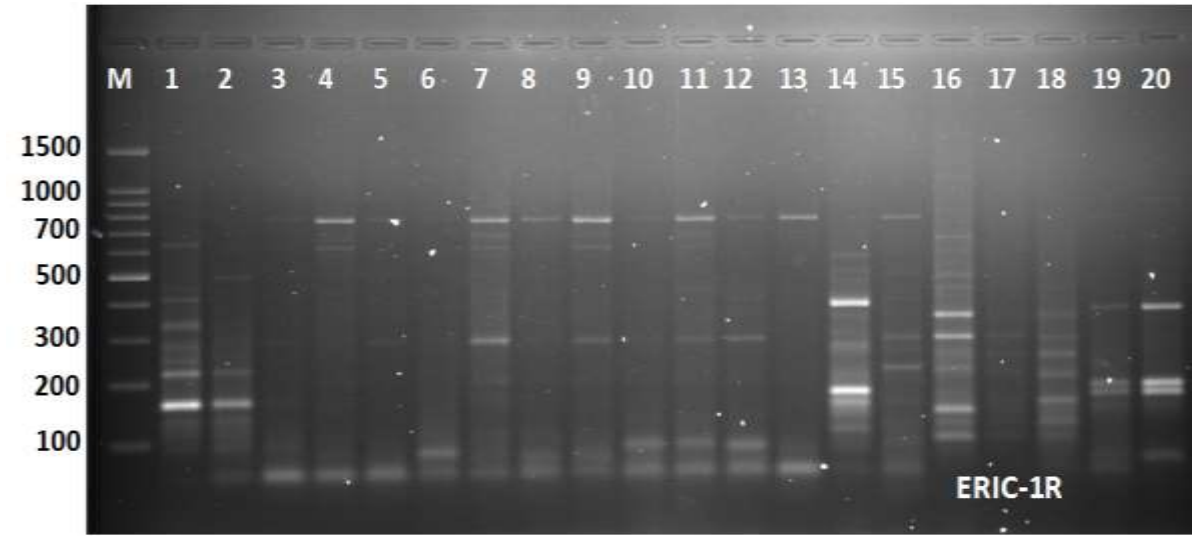

Fig. 3: Rep-PCR profile of individual samples of seven fish species generated by primer ERIC-1R. Whereas, 1-3= Morgan fish, 4-6 Mousa fish, 7-9= Hamor fish, 10-12= Shour fish, 13-15= Dennis fish, 16-18= Harid and 19-20= Black surgeon fish.

PCR amplification of ISSR markers for seven Saudi fish species.

PCR amplification results of genomic DNA for seven Saudi fish species using different DNA primers were summarized in Table 2 and shown in Fig. 2 and 3. Polymorphic and monomorphic bands were produced from the PCR amplification. ISSR markers produced 47 bands of which 38 bands were polymorphic and 9 were monomorphic. The band size ranged from 250 to 1750 bp.

PCR amplification of Rep-PCR markers for seven Saudi fish species

Results of Rep-PCR markers are presented numerically in Table 2 and captures of bands are also displayed in Fig.3. Rep-PCR markers produced 42 bands with 38 band were found to be polymorphic and only 4 bands were monomorphic. The number of total bands varied from 12 for each of REP1R-I and GTG-5, to 18, for primer ERIC1R. Band size ranged from 50 to 2100 bp. The highest polymorphism among populations was found for ERIC1R primer (100%), followed by REP1R-I primer (91.6%), where the lowest polymorphism was 75% resulted from application of GTG-5 primer.

Genetic distances and the cluster dendrogram

According to genetic similarity and intra-species differentiation and the dendrogram constructed using Neighbor Joint method based on Jaccard's similarity coefficients that ranged from 0. 57 to 0.98 (Fig 6). Phylogenetic analysis showed that genetic distance among native Saudi fish species was relatively low in general. The seven Saudi fish species were grouped into two large linages with about 59% genetic similarity. The first linage comprised only Morgan specie, while the second linage contained other Saudi Arabia fish species. This result suggested that this six species were descended from Morgan fish. The second linage comprised two clades; the first clade contained four species (Mousa, Hamor, Shour, and Dennis), where the second clade included only Harid and Black surgeon. The configuration of the first clade revealed that Hamor and Shour fishes are more closely related than Mousa and Dennies species. It could be concluded from this result that although these four species shared common ancestor, Mousa and Dennies separated earlier as independent species than the separation of Hamor and Shour.

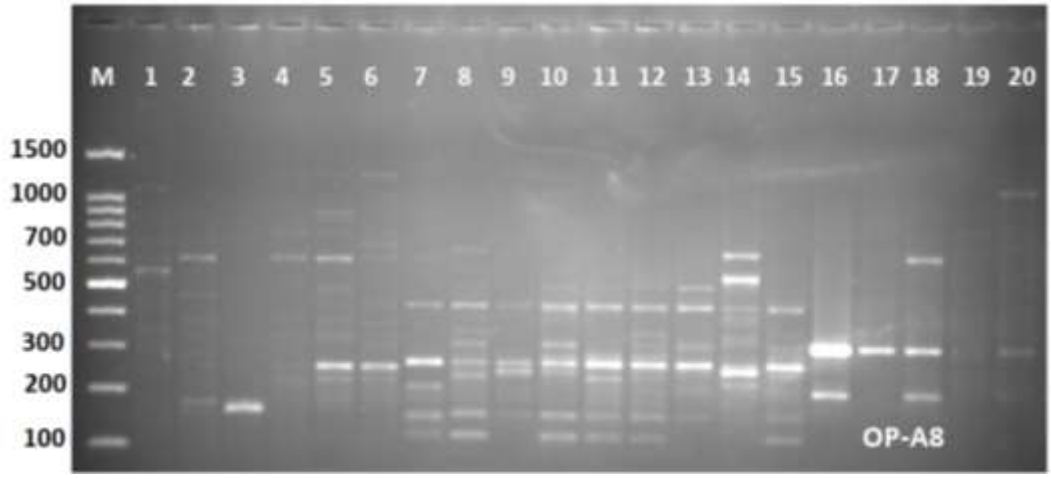

Fig. 4: RAPD profile of individual samples of seven fish species generated by primer OP-A8. Whereas, 1-3= Morgan fish, 4-6 Mousa fish, 7-9= Hamor fish, 10-12= Shour fish, 13-15= Dennis fish, 16-18= Harid and 19-20= Black surgeon fish.

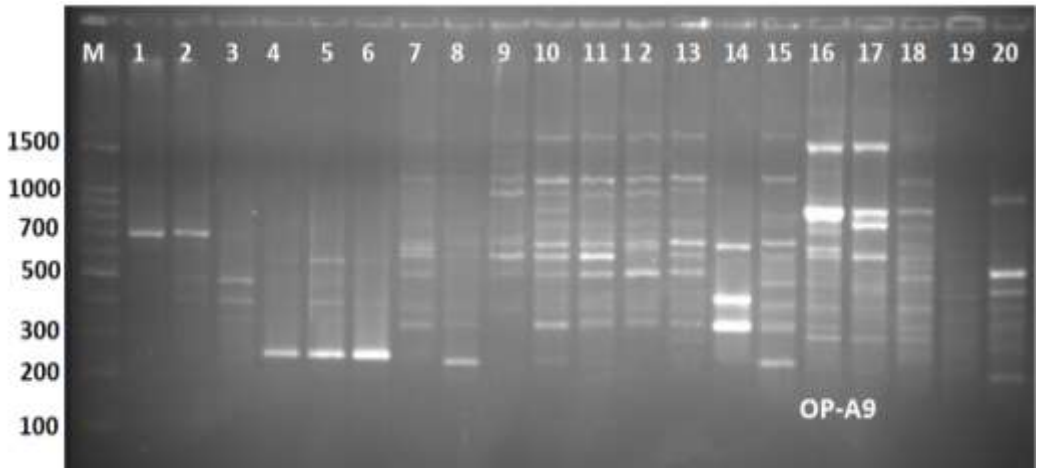

Fig. 5: RAPD profile of individual samples of seven fish species generated by primer OP-A9. Whereas, 1-3= Morgan fish, 4-6 Mousa fish, 7-9= Hamor fish, 10-12= Shour fish, 13-15= Dennis fish, 16-18= Harid and 19-20= Black surgeon fish.

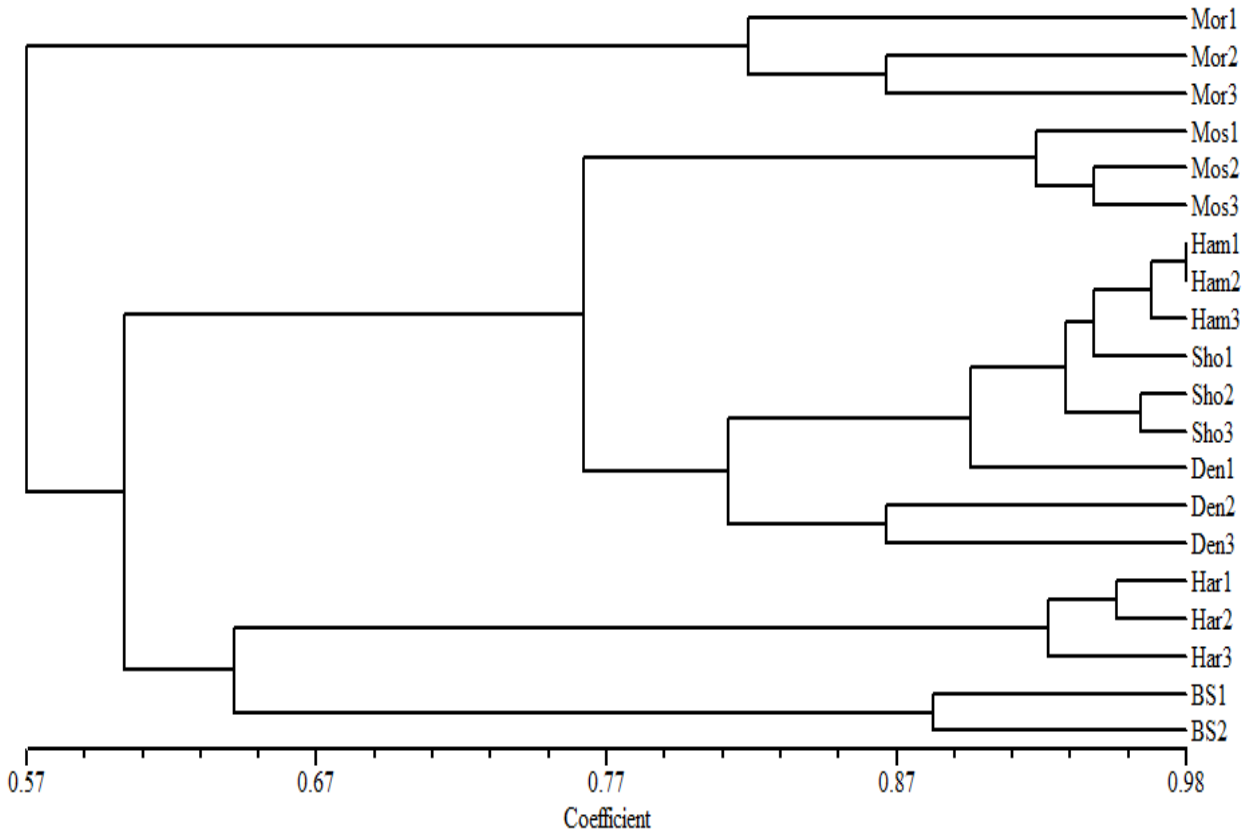

Fig. 6 Dendrogram analysis among the twenty individual samples of seven Saudi Arabia fish species based on Rep-PCR , ISSR and RAPD primers.

Discussion

To the best of our knowledge this work is breaking new ground in two directions, *first*, molecular characterization of Saudi fish, *second* employment Rep-PCR genetic marker for molecular characterization of fish species.

ISSR technique was used because it is simple and reliable tool for assessing the molecular genetic variability within and among many living organisms with highly reproducible results and abundant polymorphism (Kol and Lazebny, 2006; Lalhruaitluanga and Prasad, 2009; Saad *et al.*, 2013 and Sabir et. al., 2013). Moreover, the potential applications of ISSR analysis for diverse aims depend on the variety and frequencies of microsatellites within the specific genomes. (Chunjiang *et al.*, 2005; Hassan *et al.*, 2014). In addition, variable ISSR patterns have potentials as dominant markers for studying genetic diversity of many fishes (Tong *et al.*, 2005; Saad *et al.*, 2012). In the present study, ISSR analysis was offered some species-specific markers. The numbers of these molecular markers were varied from species to species. These DNA markers will be useful value, especially in fish breeding programs, which use genetic markers for marker-assisted selection to improve the fish quantitative traits for better porduction (Rashed *et al.*, 2009).

RAPDs were proved to be useful as genetic markers and fingerprinting (Salem *et al.*, 2005; Rabie and Abdou, 2010; Nikkhoo *et al.*, 2011; Ghanem *et al.*, 2012; Ahmed and Rezk, 2015). It can be concluded from this study that RAPD markers are effective in detecting similarity between Saudi fish species and they provide a potential tool for studying the inter-species genetic similarity and the establishment of genetic relationships. Although major bands from RAPD reactions are highly reproducible, minor bands can difficult to repeat due to random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers. The same idea was tested by Rashed *et al.*, (2011) and Hassan *et al.*, (2014). They used RAPD marker to detect the genetic variations among some fish species. The molecular genetic markers are widely used to identify lines or strains, define stock diversity, monitor inbreeding, diagnose simply inherited traits and even to improve stocks (Rashed *et al.*, 2008; Rashed *et al.*, 2009). The application of DNA-based genetic analysis as marker-assisted selection in fish research and stock development and management is still not fully maximized (Kocher *et al.*, 1998 and Rashed *et al.*, 2009). This conclusion was previously confirmed using another analysis such as RAPD. However, Rashed *et al*, (2011) and Saad *et al.* (2012) used bulked segregate analysis to reconstruct the phylogenetic relationships among three fish species. They found that *T.*

zillii species was distantly related from both *O. aureus* and *O. niloticus* species. Liu *et al.*, (2006) studied the genetic diversity in three *Paralichthys olivaceus* populations using ISSR analysis, which was confirmed to be a reproducible and sensitive tool for the study of population genetics of these fish. The genetic variability of domestic hatchery populations has implications to the conservation of natural *Paralichthys olivaceus* resources (Yun-Guo *et al.*, 2006). The use of ISSR and RAPD primers consisting of degenerate anchors or degenerate motifs increased the number of amplified markers. Since ISSR analysis is an easy to perform, high flow-through technique may represent it an alternative for the RAPD, better reproducibility was characterized due to the elevated annealing temperatures. An especially attractive feature of ISSR analysis is its flexibility in terms of experimental design, where the number of generated amplicons may be optimized by changing the number of the core repeat units and anchoring bases (Liu and Wendel, 2001; Hassan *et al.*, 2014; Ullah *et al.*, 2015). We suggest that ISSR analysis should be a stand by choice for genome mapping or gene tagging and marker-assisted selection. For its high simplicity, ISSR analysis should be the first choice for genome mapping or gene tagging for organisms (which genomic knowledge is limited). The above-mentioned exploitation and further studies would be significant for the basic and applied research on fisheries and aquaculture genetics and extend the knowledge of microsatellite conservation and evolution in Saudi fish species.

Conclusions

The effectiveness of Rep-PCR, RAPD and ISSR markers in detecting polymorphism among different Saudi Arabia fish species, their applicability in population studies and establishment of genetic relationships demonstrated with this study. It is important to mention the fact that data resulted from using these three molecular markers assays can be extended to further dissect traits in a more refined way to exactly knowledge on specific genes and genetic pathways using other molecular methodologies. There is also the opportunity and need to study sequences of specific polymorphic bands, to determine the genes detected by Rep-PCR, ISSR and RAPD experiments. Further studies using different molecular are essential to clarify and confirm genetic relationships among Saudi Arabia fish species depicted using Microsatellite Markers.

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