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GENETIC POLYMORPHISM OF *BULINUS TRUNCATUS* THE INTERMEDIATE HOST OF *SCHISTOSOMA HAEMATOBIUM* IN EGYPT USING ISSR MARKERS

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ABSTRACT

This study presents the first results about the genetic polymorphism and diversity among the five Egyptian *Bulinus truncatus* populations, Giza (GZ), Damietta (DM), Behera (BH), Fayoum (FY) and lab bred strain (LB), using the inter-simple sequence repeats PCR (ISSR-PCR). A total of 85 bands, unique, polymorphic and monomorphic were obtained using 10 ISSR primers. Out of them, 28 bands were polymorphic. Among *B. truncatus* populations, the highest polymorphism (57.1%) was revealed by the primer ISSR1, followed by that revealed by ISSR7 (40%). However, the lowest polymorphism was 16.6% and resulted from application of ISSR6 and ISSR8 primers. ISSR5, ISSR7, ISSR9 and ISSR10 primers revealed four unique bands of size 389, 199, 139 and 154 bp respectively for FY population. All bands resulted from application of ISSR4 primer were monomorphic. Genetic similarity and relationship between the five populations were detected using cluster analysis. The high genetic variability obtained from ISSR markers showed diverges of FY population from the others which may be interpreted by located Fayoum region in an isolated area from the Nile Valley. It was noted the presence of a high level of genetic similarity between GZ and LB populations, that may be due to the origin of LB was from Giza governorate. Our results have provided evidence for the usefulness of ISSR markers to analyze genetic variability among *B. truncatus* populations.

INTRODUCTION

Knowing genetic divergence of snail and schistosoma populations gives an extra dimension to pay attention and monitor of disease. Rollinson *et al*. (2009) reported that the enforcement of molecular genetics tools will be helping to improve the strategies of schistosomiasis control. Genetic divergence has been fully examined by Inter Simple Sequence Repeat (ISSR). ISSR was reported as more preferable tool than other markers for studying genetic divergence between quite near individuals (Fang *et al.*, 1997). Many studies have shown that this approach can be used as a useful tool for the genetic diversity monitoring in different populations (or breeds) of animals (AhaniAzari *et al.*, 2007; Kol and Lazebny, 2006). 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 *Bulinus* species, other molecular techniques were used by numerous researchers to study genetic diversity among different populations. For example, Eight sites in the Zimbabwean river were investigated study genetic diversity of *B. globosus* (freshwater snail) and its parasite *S. haematobium* using RAPD markers (Davies *et al*., 1999). Raahauge and Kristensen (2000) characterized the *B. africanus* group by RAPD-PCR, and ITS-RFLP. Additionally, Jones *et al*. (2001) studied the relationships among *B. forskalii* group Also by RAPD-PCR. Present work was aimed to study the genetic polymorphism and to determine the relationships between different

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populations of *Bulinus truncatus* collected from four Egyptian governorates: Giza, Damietta, Behera and Fayoum using ISSR markers.

MATERIALS AND METHODS

Snails sample collection

Four populations of *B. truncatus* snails were collected from four Egyptian governorates: Giza (GZ), Damietta (DM), Behera (BH) and Fayoum (FY), Figure (1). In addition fifth population of *B. truncatus* snails was lab bred strain (LB) obtained from Schistosome Supply biological Program (SBSP) at Theodor Bilharz Research Institute (TBRI) in Egypt.

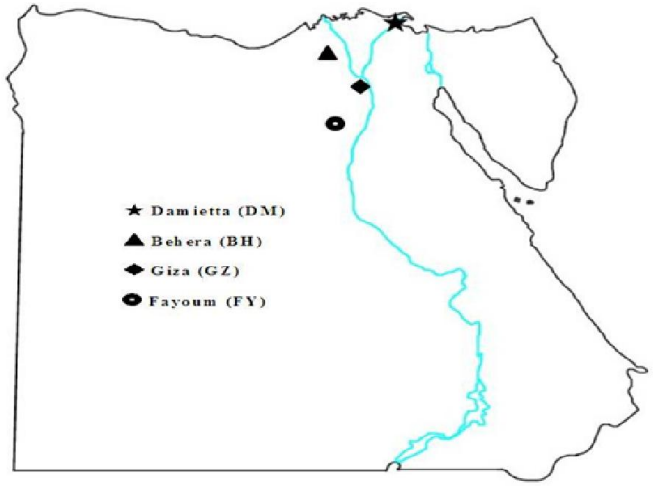

Figure 1. Egyptian map showing the collection sites of *Bulinus truncatus* populations

DNA isolation

Genomic DNA isolation from the five populations of *B. truncatus* snails were performed by Qiagen Kit (DNeasy Blood & Tissue), following the instructions of the manufacturer.

ISSR-PCR amplification

PCR was performed in 30 µl volumes tubes according to Williams *et al*. (1990) and containing the following: 3 µl DNTPs (2.5 mM), 3 µl $MgCl_2$ (25 mM), 3 µl Buffer (10 x), 2 µl Primer (10 pmol), 0.2 µl Taq DNA polymerase (250 U), 2 µl template DNA (25 ng), 16.8 µl H_2O (d.w). The PCR was programmed as follows: One cycle at 94˚C for 2 min, 45 cycles of 94˚C for 60 sec, 35˚C for 90 sec, 72˚C for 120 sec, One cycle at 72˚C for 5 min, then 4˚C for termination. Ten primers were used for ISSR analysis (Table 1).

Table 1. The ISSR primers used in the present study

Name	Code	Sequence
ISSR 1	814	(CT)8TG
ISSR 2	844A	(CT)8AC
ISSR 3	844B	(CT)8GC
ISSR 4	17898A	(CA)6AC
ISSR 5	HB 9	(GT)6GG
ISSR 6	HB 10	(GA)6CC
ISSR 7	HB 11	(GT)6CC
ISSR 8	HB 13	(GAG)3GC
ISSR 9	HB 14	(CTC)3GC
ISSR 10	HB 15	(GTG)3GC

The PCR products were run in 1.3% agarose gel; DNA ladder mix was used as standard DNA. Gels were photographed using a 35 mm color film (200 ASA) and scanned with Bio-Rad video densitometer Model 620, at a wave length of 577. Software data analysis for Bio-Rad Model 620 USA densitometer and computer were used.

Records of ISSR bands and data analysis of genetic diversity

ISSR-PCRs were performed for the five samples, and PCR products were separated in agarose gels after electrophoresis. ISSR-DNA fragments were scored as 1 or 0 for the presence or absence of bands, respectively. The obtained data were subjected to analysis with GelAnalyzer3 (Egygene) software. The level of similarity between species was established as the percentage of polymorphic bands, and a matrix of genetic similarity was compiled using Jaccard's similarity coefficient (Jaccard 1908). Similarity coefficients were used to construct the dendrogram using the unweighted pair group method with an arithmetic average (UPGMA). All calculations were performed with the NTSYS-pc 2.02 software package (Rohlf 2000).

RESULTS

PCR amplification and ISSR Analyses of *B. truncatus* Populations

The results of PCR analysis done on the genomic DNA of five *B. truncatus* populations using ten ISSR primers were summarized in table (2) and shown in Figures (2: A-J). The unique, polymorphic, and monomorphic bands were produced from the PCR amplification. A total of 85 bands were obtained from the ISSR primers. Out of them, 28 bands were polymorphic with a polymorphism average of 33%. The number of total bands varied from 6, with ISSR6, to 11, with primer ISSR4 with band size ranging from 121 to 1386 bp. The highest polymorphism among populations was revealed by ISSR1 primer (57.1%), followed by that revealed by ISSR7 primer (40%). However, the lowest polymorphism was 16.6% and resulted from application of ISSR6 and ISSR8 primers. ISSR5, ISSR7, ISSR9 and ISSR10 primers revealed four unique bands of size 389, 199, 139 and 154 bp respectively for FY population. All bands resulted from application of ISSR4 primer were monomorphic.

Genetic distances and the cluster dendrogram

The Jaccard's similarity coefficient values ranged from 0.759 to 0.988 (Table 3); showed a high relationship between Damietta and Behera of *B. truncatus* (0.988) and low genetic similarity between Giza and Fayoum (0.759). The dendrogram constructed using UPGMA based on Jaccard's similarity coefficients (Fig. 3) indicated that Damietta and Behera populations were in the same clade cluster and appeared more similar to each other than Fayoum population. Furthermore, Giza and lab bred populations were more similar to each other than other populations. The UPGMA dendrogram also showed that the Fayoum population to Giza or lab bred was did not gather together.

Table 2. Size, total number of amplified bands, number of polymorphic, monomorphic, unique bands revealed by ten ISSR primers among five snail populations of *B. truncates*

Primers	Product size (bp)	No. of amplified bands/snail population					Total no. of bands	No. of polymorphic bands	%of polymorphism	No. of Monomorphic bands	No. of unique bnds
		GZ	DM	BH	FY	LB					
ISSR1	204-1073	3	7	7	7	3	7	4	57.1	3	
ISSR2	342-986	5	6	6	7	7	7	2	28.5	5	
ISSR3	286-1386	8	9	8	8	7	9	3	33.3	6	
ISSR4	245-1262	11	11	11	11	11	11	0	0	11	
ISSR5	256-1150	6	8	8	9	6	9	3	33.3	6	
ISSR6	256-1055	5	6	6	5	6	6	1	16.6	5	
ISSR7	157-1168	7	9	9	10	7	10	4	40	6	
ISSR8	225-919	5	6	6	5	6	6	1	16.6	5	
ISSR9	121-862	7	9	9	10	7	10	3	30	7	
ISSR10	123-798	7	9	9	10	7	10	3	30	7	

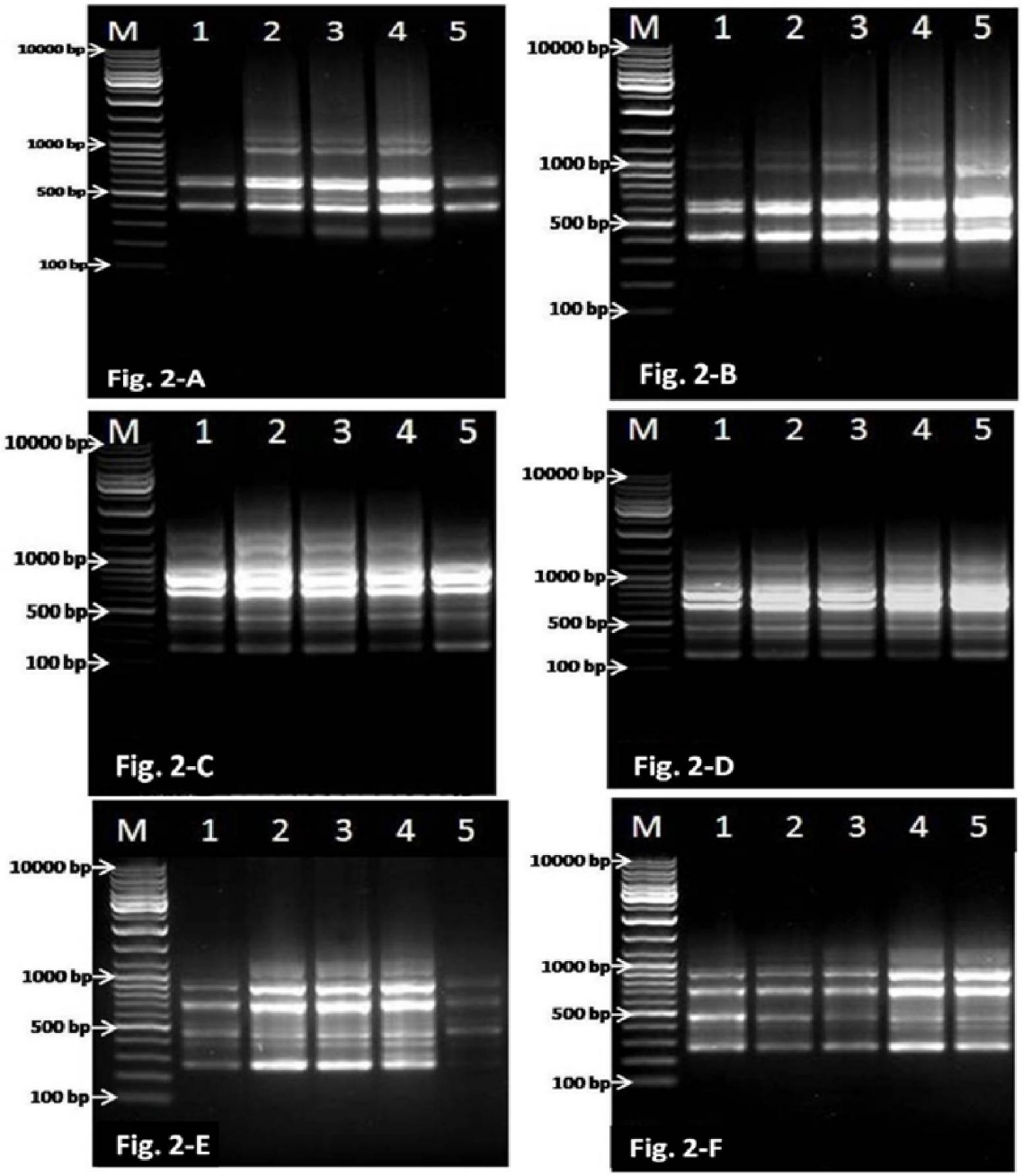

Fig. 2. Electrophoretic patterns detected in DNA of *B. truncatus* populations using ISSR primers: (A) ISSR1, (B) ISSR2, (C) ISSR3, (D) ISSR4. (E) ISSR5, (F) ISSR6,. Lan M: DNA ladder, Lan 1: Giza, Lan 2: Damietta, Lan 3: Behera, Lan 4: Fayoum and Lan 5: lab bred populations

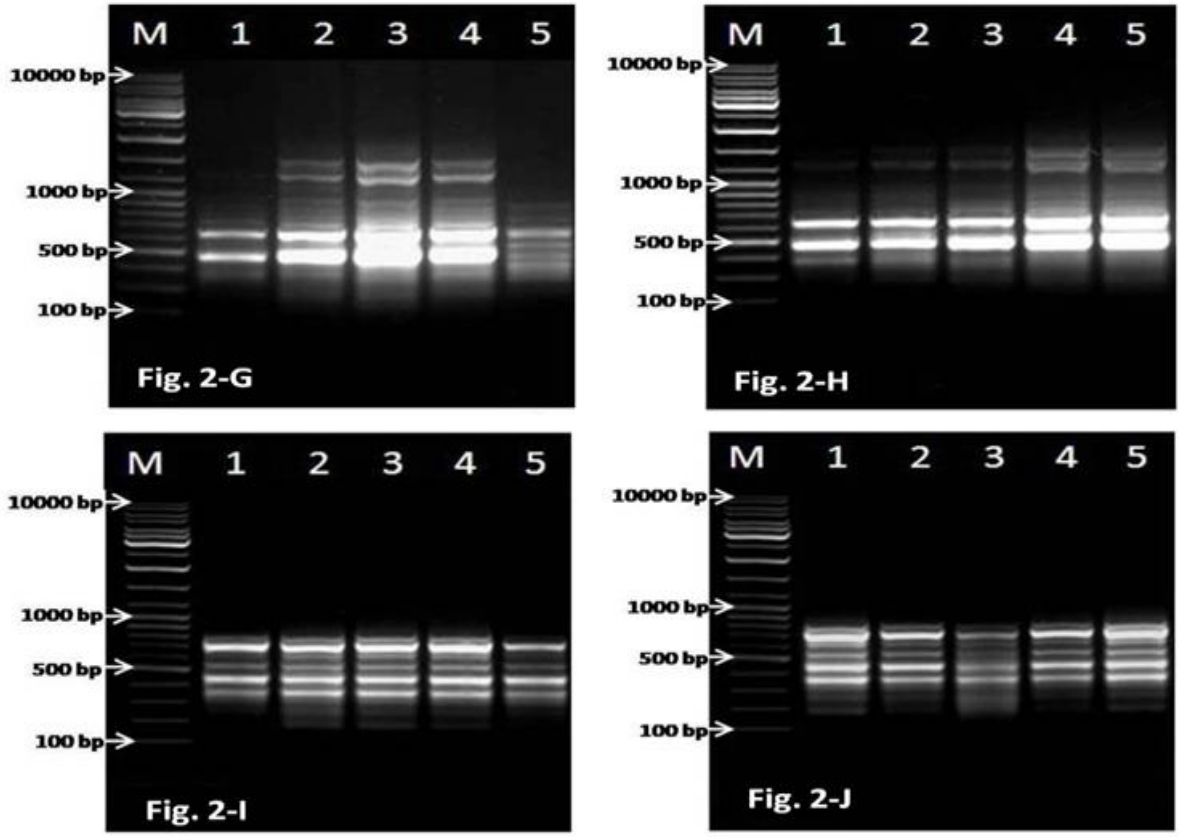

Fig. 2. Continue: Electrophoretic patterns detected in DNA of *B. truncatus* populations using ISSR primers: (G) ISSR7, (H) ISSR8, (I) ISSR9, (J) ISSR10. Lan M: DNA ladder, Lan 1: Giza, Lan 2: Damietta, Lan 3: Behera, Lan 4: Fayoum and Lan 5: lab bred populations

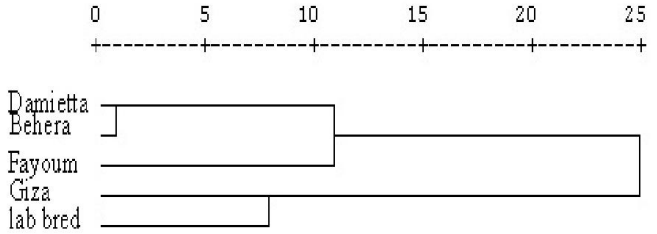

Fig. 3. Dendrogram of *B. truncatus* genotypes constructed using UPGMA based on Jaccard's similarity coefficients. Scale is a Jaccard's coefficient of similarity

Table 3. Matrix of the genetic similarity of five *B. truncatus* populations based on ISSR data analysis

Case	Giza	Damietta	Behera	Fayoum	lab bred
Giza	1.000				
Damietta	0.800	1.000			
Behera	0.810	0.988	1.000		
Fayoum	0.759	0.906	0.894	1.000	
lab bred	0.925	0.790	0.778	0.793	1.000

DISCUSSION

ISSR markers have been confirmed to be strong tools for the estimation of genetic diversity. These markers are highly polymorphic, fast, reliable, inexpensive, and are useful to discriminate even closely related genotypes. Although ISSR markers have widely been used to the study of genetic diversity in crop plants (Obeed *et al.*, 2008), populations of cattle, goat and sheep (Askari *et al*., 2011), silkworm (Kar *et al*., 2005), parasitoid (Ardeh, 2013) and microorganisms (Abadio *et al.*, 2012; Fatima *et al.*, 2012), the method using these markers has not been applied to the investigation of genetic polymorphism among *Bulinus truncatus* populations. This was therefore the first application of the ISSR technique in the investigation of genetic variation among Egyptian *B. truncatus* populations. From parasitological point of view, such genetic differences among Egyptian *B. truncatus* populations may be lead to variation in susceptibility to the infection with *S. haematobium* among them; Basch (1976) recorded that the genotypes of both partners (parasite snail and snail) detect the result of each infection. Moreover, Oliveira *et al.* (2008) alleged that sensibility or refractoriness of the snails to *S. mansoni* is relies on genetic background of the host, or results from the interaction between the gene products of the parasite and the mollusk. In addition, genetic differences in infectivity are thought to be the result of geographic adaptation of schistosomes to snails (Mulvey and Vrijenhoek, 1982). Considerable genetic differentiation among *B. truncatus* populations from different Egyptian governorates is probably associated with several factors, such as limited gene flow among these populations, and differences in environmental conditions. In this study, the greatest similarity (0.988) was obtained between Damietta and Behera populations. The reason for the narrow genetic diversity is because of these two populations we obtained from closest governorates. Moreover, ISSR analysis also revealed high level of genetic similarity between Giza and LAB populations.

This similarity may be due to that the origin of LAB population was from Giza governorate. Whereas the *B. truncatus* snails were collected from Nile River at Giza region and maintained in the laboratories of Schistosome Supply biological Program (SBSP) at Theodor Bilharz Research Institute (TBRI) in Egypt for about ten years (Fouad Yossif, personal communication). On the contrary, results of ISSR primers showed diverge of Fayoum population from the other studied populations. This magnificent genetic diversity may be due to the presence of Fayoum region in an isolated area and far away from other regions. ISSR markers give multi locus patterns, which are useful in studying genetic diversity for the most living organisms and are reflect the environmental changes (Bornet and Branchard, 2004; Sharma *et al*., 2008; Christopoulos *et al.*, 2010). Recently, ISSR-PCRs analyses were reported to be more advantages than other DNA markers. These features due to the ease, low cost, and no need for the sequence data as well as the use of long primers with high annealing temperature, which produce great band reproducibility (Jabbarzadeh *et al.*, 2010; Li *et al.*, 2010). In conclusion, DNA analysis of the ISSR markers has become a useful tool for managing genetic variability in most living organisms. Our results have provided evidence for the usefulness of ISSR markers to analyze genetic variability among *B. truncatus* populations. The study was the first application of the ISSR technique in the investigation of genetic variation among Egyptian *B. truncatus* populations.

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