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Research Article

# Molecular Approach for Detection Compatibility/Incompatibility of Egyptian *Biomphalaria alexandrina* and *Bulinus truncatus* Snails with Their Miracidia

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**ABSTRACT**

Schistosomiasis remains one of the most prevalent parasitic infections in developing countries. Understanding the molecular basis of snail-parasite interaction and identified genes that may be involved in rendering snails resistant to infection is a great challenge for disease control. The aim of the present work is to confirm, from the genetic point of view, the compatibility/ incompatibility of two types of snails; Biomphalaria alexandrina and Bulinus truncatus with Schistosoma mansoni and Schistosoma haematobium miracidia using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). We selected 7 primers, since they have previously been useful to detect polymorphism among B. alexandrina and/or B. truncatus. The electrophoretic patterns resulting from DNA amplification showed similar identify bands between each species with their compatible miracidia. No similar identify in any bands between each species with the non compatible miracidia was observed. In conclusion, there is a relatively restricted genetic variation between susceptible and non-susceptible snails; hence the susceptibility of snails to parasitic infection is possible to be genetically controlled and to develop new strategies to control schistosomiasis.

**Key words**: Schistosoma mansoni; Schistosoma haematobium; Compatibility-RAPD-PCR.

## INTRODUCTION

Schistosomiasis is a parasitic disease caused by several species of fluke of the genus schistosoma. It is the second most socioeconomically devastating parasitic disease after malaria [1]. Egypt is considered as one of the most endemic areas in the world with infection rates exceeding 80% (5-6 million) in some localities in the Nile Valley [2]. WHO recommends that a major focus of research on schistosomiasis should be on the development and evaluation of new strategies and tools for control of the disease [3]. Snail control has gained a considerable interest being easier, cheaper, safer and more promising [4]. In host–parasite compatibility, the parasite is able to find and penetrate the host and to avoid or suppress its immune responses by producing a redox equilibrium in the form of antioxidant system [5], while in resistant specimens, a strong host reaction occurs which is an expression of an innate cellular internal defense mechanism [6].

Genetic control of the snails plays an important role in schistosomiasis control [4]. A growing interest revolves around identifying the products of the snail and parasite genes influencing these associations. Previous studies have demonstrated great variability in the suitability of different snail genera and species to act as carriers for S. mansoni species [7,8].

Starting from the early 1990s, there has been an increasing interest in the developing of genetic on snail species in order to change the susceptibility of natural snail population from being predominantly highly susceptible to a non-susceptible state, through release of refractory snails into natural habitats [7, 9].

To determine which genetic components of the snail influences either the survival or destruction of a parasite infection, several isogenic snail lines have been established to display various resistant or susceptible phenotypes [10].

The random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) technique provides a screening method to identify regions of genomic amplification, deletion, or rearrangement, without the need for prior sequence information about the genome being investigated [11].

The research on genetic studies as a tool to understand host-parasite association in snails has directed our interest to study these genetic variations in two types of snails B.

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Table 1: Sequence of primers

| primer | Sequence |
|---|---|
| 1 | 5'-TGCCGAGCTG-3' |
| 2 | 5'-GGGTAACGCC-3' |
| 3 | 5'-GTG ATCGCAG -3' |
| 4 | 5'-GAAACAAATG -3' |
| 5 | 5'-ACCTACCGTACTATGACG -3' |
| 6 | 5'-GTGACGTAGG -3' |
| 7 | 5'-GTTGCCAGCC -3' |

alexandrina and B. truncatus; the intermediate hosts for S.mansoni and S. haematobium, respectively. In this concern the compatibility of each snail with its respective parasite and its incompatibility with non respective parasite was evaluated from the genetic point of view.

**MATERIALS AND METHODS**

Maintenance of snail: Biomphalaria alexandrina and Bulinus truncates snails were obtained from Schistosoma Biological Supply Project (SBSP), Theodore Bilharz Research Institute, Egypt Each species of these snails were maintained, as stock cultures, in a well-prepared snail room, under suitable environmental conditions, in glass aquaria, each containing 5L of conditioned water (pH 7.0 ± 0.2 at 25oC), at a density of 10 snails/ L [12]. The snails were fed fresh lettuce leaves supplemented with tetramin (Fish food) (Ingredient; fish meat, shrimp meat, aquatic plants, oat flour yeast, various vitamins, chlorophyll). The amount of the given food was approximately the quantity that would normally be consumed within 24 hours [13].

Selection of susceptible snails: Juvenile snails of B. alexandina and B. truncatus (3 ± 1 mm in diameter) were exposed individually to 50 freshly hatched specific miracidia in vials containing 3 ml water for 3 to 4 hours (pH=7, 25ºC) [12]. Snails susceptible to infection were examined through its cecarial shedding and isolated in well condition aquaria. Resistant snails were avoided. Self breeding of the susceptible snails to the fourth generation were isolated consequently. The fourth generation snails were selected for this study.

Experimental design- Snails were divided into two groups. Group 1 served as non infected susceptible B. alexandina snails. Group 2 served as non infected susceptible B. truncatus snails. Miracidia of S. mansoni and S. haematobium of Egyptian strains obtained from Schistosome Biological Supply Project (SBSP), Theodur Bilharz Research Institute, Egypt were used for studying the compatibility and incompatibility with the specific host.

DNA extraction: DNA was extracted from the tip of the head foot region, of individual juveniles snails of both B. alexandrina and B. Truncatus snails as well as from their miracidia of S. mansoni and S. haematobium, respectively by the method of Winnepenninckx et al. [14] with slight modification to overcome the problems associated with DNA degradation [15]. Lysis buffer containing 2% cetyl trimethyl ammonium bromide (CTAB) was used and incubated with proteinase K (2 µg/ml) for 2 h, following extraction with phenol, phenol: chloroform (1:1) and chloroform: isoamylalcohol (24:1) and incubation with ribonuclease A (350 µg/ml). The DNA was precipitated by adding isopropanol (2:3 v/v) and leaving overnight at room temperature. After centrifugation DNA was washed in 75% ethanol, 10 mM ammonium acetate for 30 min, recovered by centrifugation (12000 g) for 10 min (4ºC) and the pellet was re-suspended in TE (10 mM de Tris, 1mM EDTA, pH 8.0). DNA concentration was spectrophotometrically (Ultrospec III, Pharmacia, UK) determined at absorbance of 260 and 280 nm as well as by 2% agarose gel electrophoresis.

Spectrophotometric determination of DNA: For quantitative determination of DNA, 10 µl of the DNA sample was transferred to 990 µl deionized water in a 1 ml quartz cuvette. Absorbency was measured at 260 nm, at which absorbency of one wave length corresponds to 50 µg of double stranded DNA per ml. Ultraviolet absorbency was also used to check the purity of a DNA preparation. With a pure sample of DNA, the ratio of absorbency at 260 nm and 280 nm (A260/ A280) should be > 1.7. Regarding that, less than 1.7 indicated that DNA sample is contaminated, either with protein or with phenol and can't be amplified in PCR. The amount of ultraviolet radiation absorbed by DNA solution is directly proportional to the amount of DNA in sample [16].

Agarose gel electrophoresis of the genomic DNA: Agarose gels are used to separate large DNA fragments, ranging from ~ 0.5 to 25 Kb as described by Helling et al. [17]. Agarose gel electrophoresis of genomic DNA was done to be sure of DNA purity. Electrophoretic separation was done for the extracted DNA and for the product of PCR.

2.6. Amplification of DNA using RAPD-PCR

The genotype of both snails was determined using 18 arbitrary 10-mer primers (1-7) by RAPD-PCR (PTC 200 Peltier Thermal Cycler (MJ Research - USA).

Amplification of each individual snail DNA genome were taken using 2 ng of DNA template of a final volume of 10 µl containing 0.8 unit of Taq polymerase (Gibco, BRL, MD, USA), 200 µM of each dNTP, 1.5 mM MgCl2, 10 mM tris-HCl at pH 8.9 with 6.4 p. moles of primers (Gibco, BRL, USA). This mixture was overlaid with mineral oil. Following an initial denaturation at 95oC for 5 min, the reaction was subjected to two cycles through the following temperature profile, at 95oC for 30 sec for denaturation, 30oC for 2 min for annealing, 72oC for one min for extension, followed by 33 cycles where the annealing step

Table 2: Size in base pair of snails and its miracidia markers.

| marker | S.m | B.a | S.h | B.t | marker | S.m | B.a | S.h | B.t | marker | S.m | B.a | S.h | B.t |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1-2948 | + | | | | P4-3028 | | | + | | | | | | |
| P1-979 | + | + | | | P4-779 | | | + | + | | | | | |
| P1-700 | | | + | | P4-420 | | | | + | | | | | |
| P1-500 | + | + | | | P4-346 | | | + | + | | | | | |
| P1-363 | | | | + | P4-300 | + | + | | | | | | | |
| P1-330 | + | | | | P4-237 | + | + | | | | | | | |
| P1-237 | | + | | + | P5-3000 | | | + | + | P6-619 | | + | | |
| P2-3028 | | | + | | P5-2891 | | | | + | P6-538 | + | | | |
| P2-670 | | | | | P5-2796 | | + | | | P6-330 | + | + | | |
| P2-476 | | | | | P5-2747 | + | | | | P6-300 | | | | |
| P2-387 | + | + | | + | P5-655 | | | + | + | P7-2972 | | | + | + |
| P2-245 | + | + | | + | P5-469 | + | | | | P7-2747 | + | | + | + |
| P2-180 | | | + | | P5-431 | | | + | + | P7-779 | | | | |
| P2-138 | + | + | | | P5-387 | | + | | | P7-600 | | + | + | + |
| P3-3056 | | | + | + | P5-346 | | | + | + | P7-488 | | | | |
| P3-3028 | | | | | P5-245 | + | + | | | P7-440 | + | | + | + |
| P3-2019 | | | | + | P5-229 | | | + | | P7-355 | | | | |
| P3-1200 | | | + | + | P5-152 | + | + | | | P7-352 | | + | + | + |
| P3-916 | | | + | + | P5-115 | + | + | | | P7-269 | | | | |
| P3-719 | | | + | + | P5-57 | | + | | | P7-245 | + | + | | + |
| P3-638 | + | + | | + | P5-41 | | | + | + | P7-166 | + | + | | |
| P3-512 | | | + | | P5-31 | + | + | | | | | | | |
| P3-426 | + | | | + | P6-3000 | | | + | | | | | | |
| P3-352 | | + | | | P6-2891 | | | | + | | | | | |
| P3-253 | + | + | | | P6-965 | | | + | | | | | | |
| P3-191 | + | + | | | P6-655 | | | + | + | | | | | |

*+:Each marker was found in miraciadia ans snajl sample*
*S.m: Schistosoma mansoni, S.h: Schistosoma Heamatobium, B. a: Biomphalaria alexandrina, B, t: Bulinus truncatus*

was altered to 40oC. In the final cycle, the extension step was continued for 5 minutes. Control specimen was run simultaneously as the test without DNA genome. The samples obtained were stored at -20°C. Finally, agarose gels are used to separate PCR product.

Agarose gel electrophoresis of PCR products: Eight microliters of each DNA amplification reaction was added to 2 µl sample buffer (0.125% bromophenol blue, 40% sucrose, 0.5% sodium lauryl sulfate, 0.1 M EDT at pH 8) and the mixture was subjected to 2% agarose gel [17] at 120 volts with (0.5 mg/ml) ethidium bromide as a stain [18]. DNA was visualized using short wave Ultra Violet

Table 3: Dice's similarity coefficient of *B. alexandrina*.

| | P1 | P2 | P3 | P4 | P5 | P6 | P7 |
|---|---|---|---|---|---|---|---|
| No. of marked band in meracidia | 1 | 2 | 5 | 3 | 5 | 4 | 4 |
| No. of marked band in snails | 2 | 3 | 8 | 3 | 5 | 3 | 5 |
| No. of shared band between meracidia and snails (a) | 1 | 1 | 5 | 2 | 4 | 3 | 4 |
| No. of band in meracidia but not in snails (b) | 0 | 1 | 0 | 1 | 1 | 1 | 0 |
| No. of band in snails but not in meracidia (c) | 1 | 2 | 3 | 1 | 1 | 0 | 1 |
| Similarity coefficient (s) | 0.67 | 0.4 | 0.77 | 0.67 | 0.80 | 0.86 | 0.89 |

*S = 2 a / 2 a + b + c, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st. P 1-7: Seven primers.*

Table 4: Dice`s similarity coefficient of *Bulinus truncatus*

| | P1 | P2 | P3 | P4 | P5 | P6 | P7 |
|---|---|---|---|---|---|---|---|
| No. of marked band in meracidia | 4 | 3 | 4 | 2 | 5 | 2 | 4 |
| No. of marked band in snails | 3 | 3 | 4 | 2 | 6 | 2 | 4 |
| No. of shared band between meracia and snails (a) | 2 | 3 | 3 | 2 | 3 | 1 | 2 |
| No. of band in meracidia but not in snails (b) | 2 | 0 | 1 | 0 | 1 | 1 | 1 |
| No. of band in snails but not in meracidia (c) | 1 | 0 | 1 | 0 | 2 | 1 | 2 |
| Similarity coefficient (s) | 0.75 | 1 | 0.75 | 1 | 0.67 | 0.5 | 0.57 |

*S = 2 a / 2 a + b + c, where: a = the number of shared bands between two individuals; b = the bands present in the 1st and not in the 2nd, and c = the bands present in the 2nd and not in the 1st.*

*P 1-7: Seven primers.*

Transilluminator and photographed directly gel. Photodocumentation system (EDAS) DC 120 Zoom Digital Camera (Eastman Kodak, NY, USA) was used directly after separation.

**STATISTICAL ANALYSIS**

Data were evaluated with SPSS (Statistical Package for the Social Sciences, version 6.0.1, Chicago, IL) software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. $P < 0.05$ is the significance difference between groups. Results were expressed as mean ±S.D of snails number in each group.

Polymorphic analysis of amplified DNA fragmen

To calculate percentage band differences between snail and its miracidia, the bands observed in a given lane were compared with those in the other lanes of the same gel, as described by Vidigal et al. [19]. The similarity level was calculated using Dice coefficient and genetic distance using the Nei and Li coefficient. The estimated similarity level is based on the probability that an amplified fragment from one isolate will also be found in another [20].

**RESULTS**

Genomic DNA amplification of the compatible snail parasite: Genetic compatibility between snail and its specific parasite including B. alexandrina with S. mansoni and B. truncatus with schistosoma haematobium were determined by RAPD-PCR technique using different oligodecamers (18 arbitrary 10-mer primers). After optimization of the reaction condition, polymorphism between the snails and its miracidia was detected using 7 different oligodecamers (Table 1). All the seven primers gave amplification product which were selected on the basis of the number and frequency of polymorphism produced among snails and its miracidia. The present results revealed that the seven primers amplified a total of 50 different bands, ranging from 31bp to 2948 bp. Over all

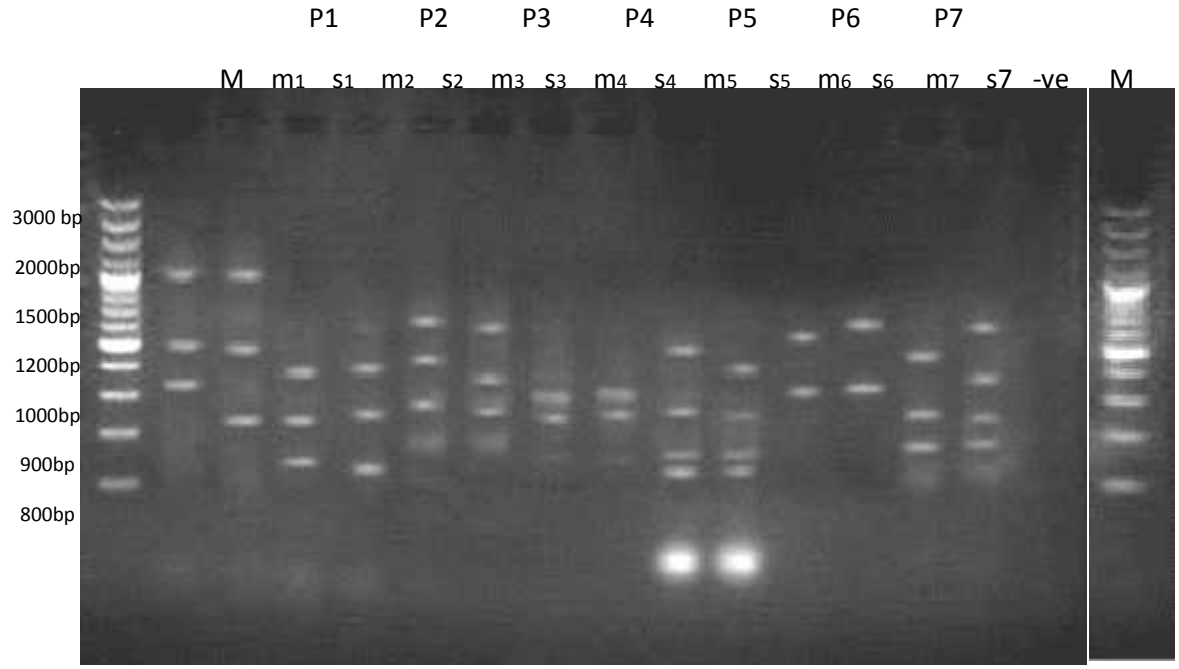


***Figure 1****: Random-amplified polymerase chain reaction from genomic DNA of susceptible B alexandrina and its miracidia S. mansoni by 7 primers visualized by 2% agarose gel and ethidium bromide staining. M : 100 bp. DNA ladder; Lanes 2-15: amplified genomic DNA of miracidia (m) and snail (s) using primer 1 (p1) to primer 7 (p7), respectively lane 16 –ve: amplified without DNA (control).*

samples, the number of RAPD bands generated per primer varied between 2 and 6 bands, with a mean of 4 bands per primer (Fig.1).

From the total 50 bands, there was a similarity in 16 bands among amplified DNA of juvenile B. alexandrina and its specific S. mansoni miracidia (Table 2). The genomic DNA, amplified with primer 1 represents similar bands between B. alexandrina with S. mansoni miracidia at 979 bp and 500 bp, primer 2 at 387 bp, 245 bp and 138 bp, primer 3 at 638 bp, 253 bp and 191 bp, primer 4 at 300 bp and 237 bp, primer 5 at 245 bp, 152bp and 115 bp, primer 6 at 330 bp and primer 7 at 245 bp and 166 bp.

Genomic DNA amplification of the non-compatible snail parasite: Figure (2) illustrates the amplification fragments in susceptible B truncatus and its miracidia; S. haematobium by 7 primers. The obtained results revealed that the seven primers amplified a total of 53 different bands, ranging from 41bp to 3028 bp. Over all samples, the number of RAPD bands generated per primer varied between 2 and 6 bands, with a mean of 4 bands per primer. From the total 53 bands, there are a similarity in 19 bands among amplified DNA of juvenile B. truncatus and its specific S. haematobium miracidia (Table 2). The genomic DNA, amplified with primer 1 presents similar bands between B. truncatus with S. haematobium miracidia at 700 bp, primer 2 at 180 bp, primer 3 at 1200 bp, 916 bp, 719 bp and 512 bp, primer 4 at 779 bp and 346 bp, primer 5 at 3000 bp, 655bp, 431bp, 346 bp and 41 bp, primer 6 at 655 bp and 300 bp, primer 7 at 2972 bp, 779 bp, 488 bp and 355 bp.

Similarity coefficient of B. alexandrina and B. truncates with their parasites: The result obtained by Dice's similarity coefficient of B. alexandrina using different primers revealed that the mean percentage of shared bands between snail and its miracidia is ranged from 0.5-0.1 (Table 3).

Dice's similarity coefficient of Bulinus truncatus using different primers expressed that the mean percentage of shared bands between snail and its miracidia is ranged from 0.67-0.89 (Table 4).

No similar identify in any bands between each species with non compatible miracidia was obtain and hence the similarity coefficient between B. alexandrina to S. haematobium or between B. truncatus to S. mansoni was zero.

## DISCUSSION

The concept of snail control on genetic basis has gained a considerable interest, to bring this hazardous disease under an adequate control. Rollinson et al. [21] reported that development of Schistosoma parasite in the intermediate host snail is influenced by a number of parasite and snail genes. The objective of disease control is to change high susceptible strains to non-susceptible state and release resistance snails into natural habitats [22]. This approach however, requires a more thorough understanding of the complex interrelationship between parasites and snails [23, 24].

The detection of specific DNA sequences by PCR has

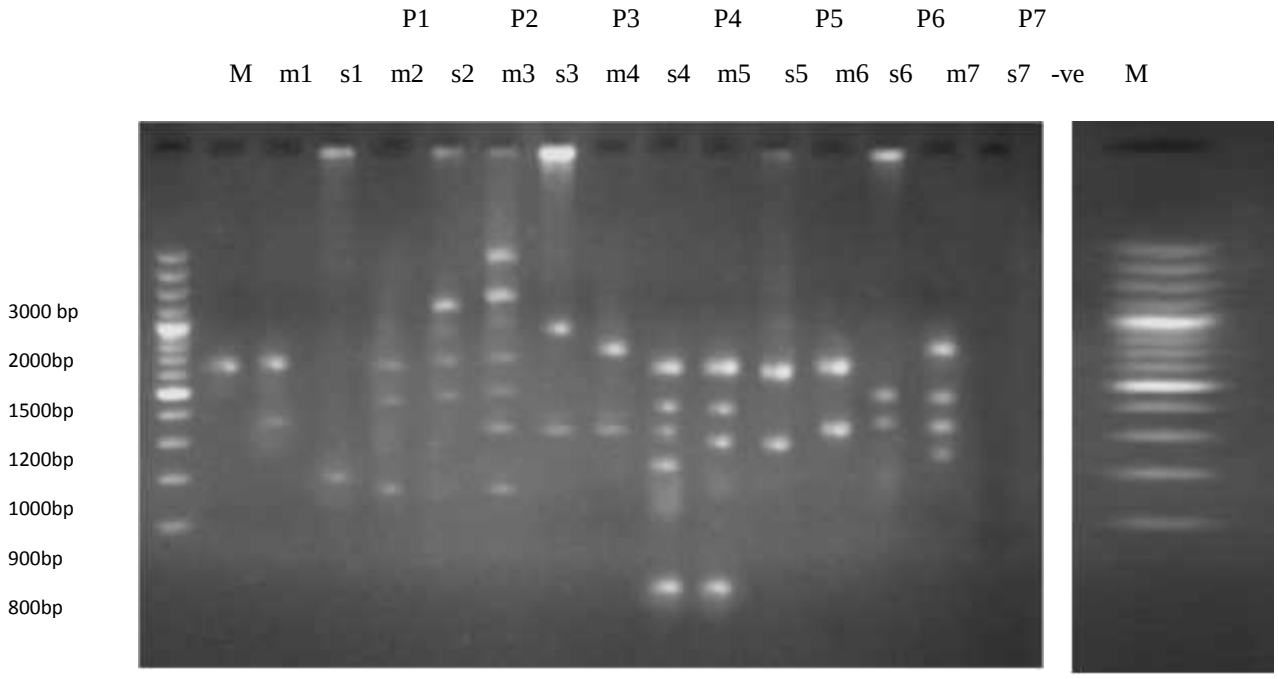


*Figure 2: Random-amplified polymerase chain reaction from genomic DNA of susceptible B. truncatus and its miracidia S. haematobium by 7 primers visualized by 2% agarose gel and ethidium bromide staining. M : 100 bp. DNA ladder; Lanes 2-15: amplified genomic DNA of miracidia (m) and snail (s) using primer 1 (p1) to primer 7 (p7), respectively lane 16 –ve: amplified without DNA (control).*

proved extremely valuable for the analysis of genetic disorders and the diagnosis of a variety of infectious disease pathogens, hence recent studies describe sensitive and specific PCR systems to detect S. mansoni, indicating possible applications in the detection of snail infection, monitoring of transmission sites, and diagnosis of human infection [25]. Abdel-Hamid et al. [15] suggested that RAPDs should be highly useful for phylogenetic analysis among closely related individuals. This suggestion is in agreement with Vidigal et al. [19] who indicated that RAPD markers are a highly resolving and helpful tool for investigation of variability. They provide a simple technology that can be used to rapidly distinguish species, strains and sexes in laboratory conditions. Also, Simpson et al. [26] proved that RAPD is undoubtedly a powerful approach for analysis of genetic variation and the identification of genetic markers.

The present work emphasizes our interest in snail genetics to determine what genes or gene products are specifically responsible for susceptibility of snails to infection. The present study demonstrated that primers from P1 to P7 showed high genetic compatibility between snails and their respective parasite which is confirmed by identification of similar genes and gene products, while they showed great variability between these parasites and the incompatible snails confirmed by the absence of common genes. The selected seven primes was in accordance with Dabo et al. [27]; Spada et al. [28] and Jamjoom [29] who studied the genetic variation between the susceptible and resistant Biomphalaria alexandrine and Bulinus truncates snails.

Berriman et al. [30] have encoded the genomic sequence of S. mansoni and showed that it consists of 11,809 genes. This study highlighted expansion for further studies on genetic schistosome /host interactions and opened the way for research on new potential targeted treatments [31].

Snail- parasite specificity in the present study showed that there is similarity in 16 bands from amplified genomic DNA of juvenile B. alexandrina snail and its specific miracidia ranged from 0.5-0.1. In addition, there is a similarity in 19 bands among amplified genomic DNA of B. truncatus and its specific miracidia ranged from 0.67-0.89. No similar identify in any bands between each species with non compatible miracidia demonstrating that the incompatibility character is based on genetic basis.

In accordance with the present work, Jannotti-Passos et al. [32] studied the snail- parasite specificity by a multiplex PCR for simultaneous identification of Brazilian Biomphalaria species and their diagnosis of infection by the trematode. The species-specific primers directed both to the internal transcribed spacer 2 of ribosomal DNA from 3 of the S. mansoni host species and to the mitochondrial DNA from the trematode, revealing the presence of specific bands efficient for identification of Biomphalaria species and diagnosis of snails infected by S. mansoni during prepatent periods.

Chen et al. [33] established a sensitive and specific PCR assay for detecting Schistosoma japonicum-infected Oncomelania hupensis, based on 18S-rRNA gene of S.

japonicum. They found the location of PCR product of detecting Oncomelania snails infected with S. japonicum was similar to the target DNA, with a length of 469 base pair and the same sequence as the target DNA.

In the present study, PCR technique was also established to determine the state of susceptibility and resistance of snails to infection. Lockyer et al. [34] identified ten transcripts, present only in the profiles derived from snails of the resistant strain when exposed to infection. Contradictory, Theron and Coustau [35] postulated that the susceptibility and resistance of Biomphalaria glabrata snails to S. mansoni infection does not depend on the snail susceptibility/resistance status, but on the 'matched' or 'mismatched' status of the host and parasite phenotypes.

Abdel- Hamid et al. [36] postulated a genetic variation between susceptible and resistant strains to Schistosoma infection within B. alexandrina snails using random amplified polymorphic DNA analysis technique, where in the resistant genotype snails, OPA-02 primer produced a major low molecular weight marker of 430 base pair.

Most studies aimed towards deciphering differences in gene regulation between resistant and susceptible snails during the snail/schistosome encounter have focused mainly on this relationship in adult, but not juvenile snails. Age dependent variability in B. glabrata susceptibility to S. mansoni has been well documented with results showing that juvenile snails (even within the same stock) are, in general, more vulnerable than their adult counterparts to infection [37].

In conclusion, the concept of genetic snail control has gained a considerable interest. Despite abundant emerging molecular information, very little is known about which snail genes to specifically target to develop transmission-blocking strategies for the eventual goal of disease control. For more permanent control of schistosomiasis, understanding of the host/parasite association is necessary, since the host-parasite relationship is complex and question remains concerning the susceptibility of snails to infection by respective trematodes and their specificity and suitability as hosts for continued parasite development. Understanding the genetics involved in the complex host/parasite relationship may lead to select actively resistant snails and mass culture them to increase the proportion of alleles for insusceptibility as a possible mean for biological control of schistosomiasis in natural population. The present results suggest that RAPD-PCR represents an efficient means of genome comparison. Many molecular markers were detected as genetic variations between susceptible and non-susceptible snails with the different parasites. Since there is a relatively restricted genetic variation between susceptible and non-susceptible snails, it is possible that the susceptibility of snails to parasitic infection is genetically controlled.

Competing interests: The authors declare that no competing of interests.

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