Genetic Variability among Four Egyptian Sheep Breeds Using Random Amplified Polymorphic DNA (RAPD) and PCR-RFLP Techniques


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Abstract: Genetic variability among four Egyptian sheep breeds (Barki, Rahmani, Ossimi and Romanov) were studied using both random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) techniques. Sixteen random primers were used to amplify DNA fragments in these four sheep breeds. RAPD patterns with a level of polymorphism were detected among breeds. The results showed that the genetic similarity among these four breeds was as follows: 90% (Barki x Ossimi), 87% (Barki x Rahmani), 80% (Ossimi x Romanov), 80% (Rahmani x Romanov) and 74% (Barki x Romanov). However, the closer proximity or the highest genetic similarity was observed between Barki and Ossimi (90%), while the lowest was observed between Barki and Romanov (74%). On the other hand, the results of the RFLP for 18S rRNA gene technique showed that, no genetic variation were found among the four sheep breeds under study. Finding in this study confirms the phenotypic classification of Egyptian sheep to three related breeds (Barki, Ossimi and Rahmani) and the other breed (Romanov) is relatively remote.

Key words: sheep, RAPD-PCR, PCR-RFLP, phylogeny, genetic similarity

INTRODUCTION

Sheep represents an important part of animal husbandry in Egypt and in many another countries. It is economically a very important farm animal and genetic improvement of these animals is of economic importance, especially in reproductive performance and quantity of meat and wool.

Based on the phenotypic characters, Egyptian sheep were classified to more than five breeds, some of them are: Barki, Rahmani, Ossimi and Romanov. To identify the genetic similarity among these four breeds, random amplified polymorphic DNA (RAPD) and polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) techniques were used in this study.

Application of the random amplified polymorphic DNA technique has greatly increased the ability to understand the genetic relationships within species at the molecular level. Information on genetic relationships in farm animals within and between species has several important applications for genetic improvement and in breeding programs.

MATERIALS AND METHODS

Animals. In the present study, forty sheep represents four sheep breeds (Barki, Rahmani, Ossimi and Romanov) in Egypt were precisely selected from three different regions. Ten female individuals from each breed were used for this study. All the animals were apparently healthy and free from any clinical disorders or diseases.

DNA isolation. For DNA isolation, forty female blood samples from previous four sheep breeds (ten from each breed) were collected. DNA extraction was carried out according to, as follows: blood samples were mixed with EDTA as and stored at -20°C. 700 µl of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µg of proteinase K (20 mg/ml) were added to 100 µl thawed blood. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform - isoamylalcohol (25:24:1) and chloroform-isoamylalcohol (24:1). DNA was precipitated by adding two equal volumes of chilled ethanol in the presence of 10% 3 M sodium acetate.
The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in 100 µl TE-buffer until needed.

**PCR and gel electrophoresis.** According to 29,14, PCR was performed in a reaction volume of 25 µl using 25 ng of pooled genomic DNA of each breed, 25 pmol of each primer, 10X Taq DNA polymerase buffer including MgCl₂, 0.2 mM dNTPs and 5 unit/µl Taq DNA polymerase (Sib-Enzyme, Russia). Thermal cycling (Perkin Elmer 9700 and Mastercycler gradient) was carried out by initial denaturation at 94°C for 4 min, followed by 40-45 cycles each at 94°C for 30-60s, annealing temperature at 28-45 for 30-60s (Table 1), polymerization temperature at 72°C for 1 min and final extension at 72°C for 10 min., then the samples were held at 4°C. The amplified DNA fragments were separated on 2-3% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

**RFLP for 18S rRNA gene.** Amplified PCR products (600 bp) from 18S rRNA gene were digested with ten different restriction endonuclease (R.E.s.): Ava, AluI, Apal, AatII, BamHI, CfoI, Cail, EcoRI, EcoRV and Hinfl. Where, one unit is defined as the amount of enzyme required to digest 1 µg of DNA for 1 hour (first four R.E.s.) and 4 hours (the other six R.E.s.) at 37°C in a total reaction volume of 50 µl. DNA fragments were separated on 2.5% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed.

**Scoring and data analysis of RAPDs.** The DNA bands were scored for their presence (1) or absence (0) in the RAPD profile of the four breeds. The index of similarity between each two breeds was calculated using the formula: Bab = 2 N_ab / (N_a + N_b), where N_ab is the number of common fragments observed in individuals a and b breeds, and N_a and N_b are the total number of fragments scored in a and b, respectively. The Band sharing (BS) values were calculated for each primer separately and average for all primers was carried out with each comparison. Dendrogram was constructed to estimate genetic similarity among four sheep breeds (Barki, Rahmani, Ossimi and Romanov) using the average linkage between group statistical systems by Statistica (5, 1995) program.

**RESULTS AND DISCUSSION**

**Results:** Sixteen random and 18S rRNA gene primers (Table 1) were used to identify the genetic similarity among four Egyptian sheep breeds: Barki, Rahmani, Ossimi and Romanov. All 16 primers, in addition to 18S rRNA gene primer, were successfully amplified on the genomic DNA from pooled samples of each breed separately. Figure 1 shows the polymorphic bands of the third random primer (as an example) among the four breeds under study, whereas PCR amplification of the gene encoding 18S rRNA in Barki, Ossimi, Rahmani and Romanov breeds generated fragment of 600 bp, as shown in Figure 2.

RAPD analysis was used to construct the parsimony tree depicting relationships among the four breeds studied (Figure 3). Data presented in Table 2 show estimated genetic similarity among four sheep breeds which was 90% (Barki x Ossimi), 87% (Barki x Rahmani), 87% (Ossimi x Rahmani), 80% (Ossimi x Romanov), 80% (Rahmani x Romanov) and 74% (Barki x Romanov).

**Discussion:** Sheep breeds were phenotypically classified to many breeds, distributed in many places in Egypt. However, in the present study, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) for 18S rRNA gene techniques were used for construction the genetic similarity among four Egyptian sheep breeds (Barki, Ossimi, Rahmani and Romanov) and for confirmation of their phenotypic classification.

As can be seen, the results in Figure 3 and Table 2 showed the genetic similarity among studied four Egyptian sheep breeds (Barki, Ossimi, Rahmani and Romanov) which was 90% (Barki x Ossimi), 87% (Barki x Rahmani), 87% (Ossimi x Rahmani), 80% (Ossimi x Romanov), 80% (Rahmani x Romanov) and 74% (Barki x Romanov). As a consequence, the closer proximity or the highest genetic similarity was observed between Barki and Ossimi (90%), while the lowest was observed between Barki and Romanov (74%). On the other hand, the results of the RFLP for 18S rRNA gene technique showed that, no genetic variation were found among the four sheep breeds under study. Finding in this study confirms the phenotypic classification of Egyptian sheep to three related breeds (Barki, Ossimi and Rahmani) and the other breed (Romanov) is distal from them as expected.

In a previous study on Egyptian sheep breeds, RAPD technique has also been used for constructing phylogenetic relationships. Where, nineteen random primers were used to amplify DNA fragments in Baladi, Barki, Rahmani and Suffolk5. The author found that the highest genetic similarity was between Barki and Rahmani (95.7%), while the lowest was between Baladi and Suffolk (81.9%).
### Table 1: List of the random and 18S rRNA gene primers, their nucleotide sequence, GC content and annealing temperatures.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequence 5' - 3'</th>
<th>G+C content %</th>
<th>Annealing temperature/ time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CGA GCC CTT CCA GCA CCC AC</td>
<td>70</td>
<td>45/60</td>
</tr>
<tr>
<td>2</td>
<td>TGC CGA GCT G</td>
<td>70</td>
<td>45/60</td>
</tr>
<tr>
<td>3</td>
<td>AAT CGG GCT G</td>
<td>60</td>
<td>45/60</td>
</tr>
<tr>
<td>4</td>
<td>GAA ACG GGT GGT GAT CGC AG</td>
<td>60</td>
<td>40/60</td>
</tr>
<tr>
<td>5</td>
<td>CGC TGT CGC C</td>
<td>80</td>
<td>40/60</td>
</tr>
<tr>
<td>6</td>
<td>AGT CCT CGC C</td>
<td>70</td>
<td>32/60</td>
</tr>
<tr>
<td>7</td>
<td>GAA TGC GAC G</td>
<td>60</td>
<td>32/60</td>
</tr>
<tr>
<td>8</td>
<td>ATG ACG TTG A</td>
<td>40</td>
<td>32/60</td>
</tr>
<tr>
<td>9</td>
<td>CTG ACG AGT G</td>
<td>60</td>
<td>45/60</td>
</tr>
<tr>
<td>10</td>
<td>GGG CTA GGG T</td>
<td>70</td>
<td>40/60</td>
</tr>
<tr>
<td>11</td>
<td>ACC GGG AAC G</td>
<td>70</td>
<td>34/60</td>
</tr>
<tr>
<td>12</td>
<td>AGC ACG TGG A</td>
<td>60</td>
<td>32/60</td>
</tr>
<tr>
<td>13</td>
<td>AGG CCC CTG T</td>
<td>70</td>
<td>28/60</td>
</tr>
<tr>
<td>14</td>
<td>ATG CCC CTG T</td>
<td>60</td>
<td>28/60</td>
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<tr>
<td>15</td>
<td>AAA GCT GCC G</td>
<td>60</td>
<td>28/60</td>
</tr>
<tr>
<td>16</td>
<td>ACC GCC GAA G</td>
<td>70</td>
<td>28/60</td>
</tr>
</tbody>
</table>

**18S rRNA**

GCA AGT CTG GTG CCA GCC (Forward) 56/60

CTT CGG TCA ATT CCT TTA AG (Reverse)

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**Fig. 1:** An example of RAPD patterns in the four Egyptian sheep breeds obtained with the third random primer in Table 1. Lane M: DNA marker (100 bp ladder), lane B: Barki, lane O: Ossimi, lane Ra: Rahmani and lane Ro: Romanov.

However, in our study, a highly genetic similarity (87%) between Barki and Rahmani breeds was also found. The RAPD technique has also been used for constructing phylogenetic relationships in other farm animals such as; foreign sheep[22,24,9,27,16,23], buffalo[1], cattle[17,13,28,15,18,25,31,5,9,6], goat[2,8,19] and horse[7].

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**Fig. 2:** Agarose gel electrophoresis of amplified 18S rRNA gene (600 bp). Lane B is Barki, lane O is Ossimi, lane Ra is Rahmani, lane Ro is Romanov and lane M is a molecular weight marker (100-bp ladder).

### Table 2: Jaccard's similarity coefficients between the four Egyptian sheep breeds based on RAPD data.

<table>
<thead>
<tr>
<th>BREED</th>
<th>Barki</th>
<th>Ossimi</th>
<th>Rahmani</th>
<th>Romanov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barki</td>
<td>--</td>
<td>90</td>
<td>87</td>
<td>74</td>
</tr>
<tr>
<td>Ossimi</td>
<td>90</td>
<td>--</td>
<td>87</td>
<td>80</td>
</tr>
<tr>
<td>Rahmani</td>
<td>87</td>
<td>87</td>
<td>--</td>
<td>80</td>
</tr>
<tr>
<td>Romanov</td>
<td>74</td>
<td>80</td>
<td>80</td>
<td>--</td>
</tr>
</tbody>
</table>
In conclusion, this study has displayed that genetic similarity exists among the four Egyptian sheep breeds (Barki, Ossimi, Rahmani and Romanov), especially among the three breeds Barki, Ossimi and Rahmani (Figure 3).

![Dendrogram using average linkage based on RAPD data analysis among four Egyptian sheep breeds.](image.png)

**Fig. 3**: Dendrogram using average linkage based on RAPD data analysis among four Egyptian sheep breeds. Where, Ba: Barki, Os: Ossimi, Ra: Rahmani and Ro: Romanov.

With further experimentations, the RAPD profile generated for each breed can be effectively used as a supporting marker for taxonomic identification. In taxonomic and molecular systematic, species-specific RAPD markers could be an invaluable tool for species variation and establishing the status of organisms and its evolution.

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**REFERENCES**