GENETIC DIVERSITY AMONG GUINEA FOWL AND A SUDANESE DOMESTIC FOWL DETECTED BY PCR-BASED RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) FINGERPRINT

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Abstract

The randomly amplified polymorphic DNA (RAPD) technique was used to estimate the genetic diversity among two subspecies of guinea fowl; tufted (Numida meleagris meleagris) and helmeted (Numida meleagris galeata) and a local breed of domestic fowl (Gallus gallus domesticus) in Sudan. Four random primers (OPM-8, OPM-10, OPP-9 and OPP-10) were selected to be used in the present investigation. Sizes of the PCR product resulted from OPM-8 showed 650-1700 bp, that of OPM-10 was 350-1400 bp, that of OPP-9 was 300-2400 bp while that resulted from OPP-10 was 200-2650bp. The index of similarity between each of the two subspecies of guinea fowl was calculated. The similarity index between the two subspecies of guinea fowls was (0.930), and between helmeted guinea fowl and domestic fowl was (0.149) whereas it was (0.125) between tufted guinea fowl and domestic fowl.

Introduction

Sudan is a home for large numbers of most kinds of animals, it is maintained under the migratory system of husbandry to make full use of seasonal fluctuation in pasture and water availability. Poultry participates by 4% of the animal resources in the gross domestic production (MARF, 2002). The indigenous breeds in the Sudan are called Baladi which include three types as recorded by Desai, (1962), as follows Large baladi, the Betwill and Bare neck birds, the birds keeping in rural areas around the homestead and no distinct system of poultry production is followed (MARF, 2002).
In Sudan, guinea fowl is found in low rainfall woodland Savanna. Three races of guinea fowl were reported to inhabit the Sudan (Cave and MacDonald, 1955). Tufted guinea fowl (\textit{Numida meleagris meleagris}) is a game bird widely distributed in the Sudan (Figure 1). The domesticated strain of the guinea fowl (\textit{Numida meleagris galeata}) was obtained from the grey-breasted or helmet guinea fowl (Figure 2).

Randomly amplified polymorphism of DNA (RAPD) has been used to detect specific markers to estimate genetic relatedness among various poultry species (Williams et al. 1990), breeds, as well as for genome mapping (Smith et al., 1995; Romanov and Weigend 2001, Singh and Sharma 2002). But, this is the first time to be performed for Sudanese birds.

**Materials & Method**

**Blood samples**

A total of one hundred and eight blood samples (2ml) whole blood was collected from the wing vein from the two varieties of guinea fowl, {tufted guinea fowl [N=40] (\textit{Numida meleagris meleagris}) and helmeted guinea fowl [N=20] (\textit{Numida meleagris galeata})} and one of local breed [N=48 {21 males and 27 females}] (Large baladi), in a vacutainer tubes containing EDTA as anticoagulant, then stored at 4°C.

**DNA extraction**

The genomic DNA samples were isolated using a protocol described by Sambrook and Russell (2001). The concentration of the DNA was determined using spectrophotometer based on absorbance at 260 and 280 nm.

**RAPD-PCR**

In the current study, ten base long oligonucleotide primers (12 primers) from Operon Technologies sc were used. Primers were randomly selected on the basis of GC content of either 60\% or 70\% viz. OPA-6,7,17 and OPM-8,9,10 and OPP-3,5,9,10 and OPB-19 and OPI-5 for RAPD-PCR amplification.

The PCR reactions were carried out in a 35\(\mu\)l volume in PCR tubes containing 60 ng of genomic DNA, 0.6 \(\mu\)l of each primer (10 pmol/ \(\mu\)l), 0.2 U/ \(\mu\)l of Taq DNA polymerase, 0.1 mM dNTP-mix, and 2.5 \(\mu\)l 10x
PCR-buffer. The thermocycler was programmed as follows: 15 min at 95 °C, 40 cycles of 1 min at 94 °C, DNA 1 min at 36 °C annealing phase, and 2 min at 72°C followed by extension of 10 min at 72 °C. The PCR products were analyzed by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. The amplified fragments were visualized on a UV transilluminator and photographed.

**Data analysis**

The RAPD bands were scored for their presence (1) or absence (0). The index of similarity between each two strains was calculated using the formula:

\[ Bab = \frac{2 \times Nab}{Na + Nb} \]

Where \( Nab \) is the number of common fragments observed in individuals \( a \) and \( b \), and \( Na \) and \( Nb \) are the total number of fragments scored in \( a \) and \( b \), respectively. The similarity index was calculated for each primer separately, and the average for all primers was carried out with each comparison (Lynch, 1990).

![Tufted guinea fowl](Image)

**Fig. 1:** Tufted guinea fowl (*Numida meleagris meleagris*).
Fig. 2: Helmet guinea fowl (*Numida meleagris galeata*).

Fig. 3: Large Baladi chicken
Results

The four primers (OPM-8, OPM-10, OPP-9 and OPP-10) used in this study yielded individual genomic DNA samples of each variety (Figs.4, 5, 6, 7). A total of 1087 scorable bands were generated. The characteristics of the fragments generated by the array of 4 random primers are shown in table (1). Depending upon the primers used the number of amplification products resolved varied between 5 and 14 bands and their molecular weight ranged from 200 - 2650 base pairs.

The similarity index between the two varieties of guinea fowls is (0.930), and between *Numida meleagris galeata* and Large baladi (0.149) and between *Numida meleagris meleagris* and Large baladi (0.125) as shown in table (2).

![PCR product of the amplified fragments of Tufted and Helmet guinea fowl and Large baladi chicken (male & female) using primer OPM-8.](image)

**Fig. 4:** PCR product of the amplified fragments of Tufted and Helmet guinea fowl and Large baladi chicken (male & female) using primer OPM-8.

- **M** = Molecular marker weight (2645-100) bp.
- **H** = Helmet guinea fowl (*Numida meleagris meleagris*)
- **T** = Tufted guinea fowl (*Numida meleagris galeata*)
- **FB** = Female of Large baladi chicken
- **MB** = Male of Large baladi chicken
Fig. 5: PCR product of the amplified fragments of Tufted and Helmet guinea fowl & large baladi chicken (male & female) using primer OPP-10.

M = Molecular marker weight (2645-100) bp.
T = Tufted guinea fowl (Numida meleagris galeata)
H = Helmet guinea fowl (Numida meleagris meleagris)
FB = Female of Large baladi chicken.
MB = Male of Large baladi chicken
Fig. 6: product of the amplified fragments of Tufted and Helmet guinea fowl and Large baladi chicken (male and female) using primer OPP-9.

M = Molecular marker weight (2645-100) bp.
T = Tufted guinea fowl (*Numida meleagris galeata*)
H = Helmet guinea fowl (*Numida meleagris meleagris*)
FB = Female of Large baladi chicken.
MB = Male of Large baladi chicken
Fig. 7: PCR product of the amplified fragments of Tufted and Helmet guinea fowl and Large baladi chicken (male and female) using primer OPM-10.

M = Molecular marker weight (2645-100) bp.
FB = Female of Large baladi chicken.
H = Helmet guinea fowl (Numida meleagris meleagris)
MB = Male of Large baladi chicken
T = Tufted guinea fowl (Numida meleagris galeata)

Table 1. PCR results of four random primers used.

<table>
<thead>
<tr>
<th>Primer Code</th>
<th>Sequences 5’to 3’</th>
<th>No of bands</th>
<th>Ranged alleles size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPM-8</td>
<td>TCTGGCGGCAC</td>
<td>5</td>
<td>650-1700</td>
</tr>
<tr>
<td>OPM-10</td>
<td>TCTGTTCGCAC</td>
<td>9</td>
<td>350-1400</td>
</tr>
<tr>
<td>OPP-9</td>
<td>GTGGGTCGCA</td>
<td>7</td>
<td>300-2400</td>
</tr>
<tr>
<td>OPP-10</td>
<td>TCCCGCTAC</td>
<td>14</td>
<td>200-2650</td>
</tr>
</tbody>
</table>
Table 2. Genetic similarity estimated between populations from the amplified pattern of selected primers.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Between population similarity (BS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numida meleagris meleagris,</td>
<td>0.930</td>
</tr>
<tr>
<td>Numida meleagris galeata</td>
<td></td>
</tr>
<tr>
<td>Numida meleagris meleagris,</td>
<td>0.125</td>
</tr>
<tr>
<td>Large baladi</td>
<td></td>
</tr>
<tr>
<td>Numida meleagris galeata,</td>
<td>0.149</td>
</tr>
<tr>
<td>Large baladi</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The RAPD technique used to measure the inter-variety genetic variation; using band sharing (BS) showed a high degree of genetic similarity in the two verities of guinea fowls (0.00 to 0.46), and low between the two guinea fowls and the Large baladi chicken (0.90 to 0.98). Similar results were obtained by Nahashon et al., (2003) for guinea fowl. The RAPD of genomic DNA seem to reveal more polymorphisms within guinea fowls, in addition it also reveal high genetic diversity between chicken and guinea fowl populations (Nahashon et al., 2003).

Results of the present work confirm the applicability of RAPD technique for genetic analysis in chicken. It provides evidence of this technique's use to evaluate genetic diversity and develop markers in guinea fowls. The disadvantages of RAPD include dominance of detected markers and difficulty of defining alleles as also reported by (Lynch., 1988). However the ease and rapidity of RAPD make it attractive for routine genetic typing. The guinea fowl genome is among the least understood of economically important poultry species.

**Acknowledgement**

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(MARF)

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