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Genetic Similarity Among the Three Egyptian Water Buffalo Flocks Using RAPD-PCR and PCR-RFLP Techniques

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Abstract: Genetic similarity and polymorphisms among the three Egyptian water buffalo flocks (Beheiry, Minoufy and Saidy) were studied using both random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) techniques. Ten random primers were used to amplify DNA fragments in these three flocks. RAPD patterns with a level of polymorphism were detected among flocks. The results showed that the highest genetic similarity was observed between Beheiry and Minoufy (85%), while the lowest was observed between Beheiry and Saidy (74%). In addition to RAPD technique, restriction fragment length polymorphism for 18S rRNA gene was also applied in this study to detect the genetic similarity among these three genotypes (Beheiry, Minoufy and Saidy) which was 100%, approximately. Finding of this study confirms the classification of Egyptian water buffaloes to three flocks according to their phenotypic characters.

Key words: water buffalo, RAPD-PCR, PCR-RFLP, phylogeny, genetic similarity

INTRODUCTION

The water buffalo represents an important part of animal husbandry in Egypt. The estimated herd number exceeds 3.6 million heads[10]. 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 milk as well as diseases and parasite resistance[9].

Based on the phenotypic characters, Egyptian water buffaloes were classified to three flocks; Beheiry, Minoufy and Saidy[8]. To identify the genetic relationship among these three flocks, random amplified polymorphic DNA (RAPD) technique was used in this study.

Application of the random amplified polymorphic DNA technique have greatly increased the ability to understand the genetic relationships within species at the molecular level. However, information on genetic relationships in livestock within and between species has several important applications for genetic improvement and in breeding programs[23].

MATERIALS AND METHODS

Animals: Fifteen water buffaloes represent the three Egyptian flocks (Beheiry, Minoufy and Saidy) were carefully selected from three different regions in Egypt. For DNA extraction, five animals from each flock (Beheiry, Minoufy and Saidy) were chosen from Beheira (Kafr El-Dawar), Minoufia and Sohag, respectively.

DNA Extraction: For DNA extraction, blood samples from Beheiry and Saidy buffaloes were collected, while hair samples from Minoufy buffalo were collected. DNA extraction was carried out by method of Sharma *et al.,*[25] as fellows: Venous blood samples were mixed with EDTA as anticoagulant and stored at -20°C. To an aliquot of 100 µl blood (after thawing), 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. 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 a high concentration of salts (10% 3 M sodium acetate). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume of ddH_2O. According to Pfeiffer *et al.,*[22], DNA was extracted from hair as follows: amounts of 10, 30 and 50 basal hair segments of approximately 15 mm length including roots were digested in 340 µl extraction buffer containing 100 mM Tris-Hcl (pH 8.0), 100 mM NaCl, 3 mM $CaCl_2$, 2% SDS (w/v), 40 mM DTT and 250 µg/ml proteinase K. The hairs were incubated at 56 °C

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Table 1: List of the random and 18S rRNA gene primers, their nucleotide sequence and annealing temperatures.

Primer	Sequence 5' → 3'	Annealing temperature °C/time (s)
1	ATG CCC CTG T	28/30
2	AAA GCT GCG G	28/30
3	ACC GCC GAA G	28/30
4	CGC TGT CGC C	40/30
5	GAA TGC GAC G	40/30
6	GGA CTG GAG TGG TGA CGC AG	54/30
7	AGG CCC CTG T	28/30
8	CAG GCC CTT CCA GCA CCC AC	50/30
9	GGT GAC GCA GGG GTA ACG CC	52/30
10	CTG AGG AGT G	40/30
18S rRNA	GCA AGT CTG GTG CCA GCC (Forward) CTT CCG TCA ATT CCT TTA AG (Reverse)	54/60

Table 2: Jaccard's similarity coefficients between the three flocks of Egyptian buffalo based on RAPD data.

Flock	Beheiry	Minoufy	Saidy
Beheiry	-	85	74
Minoufy	85	-	81
Saidy	74	81	-

for 2-5 h. The DNA was purified and washed using the same steps of DNA extraction from blood.

PCR and Gel Electrophoresis: According to Williams *et al.,*[27] and Kuske *et al.,*[13], PCR was performed in a reaction volume of 25 µl using 25 ng of genomic DNA from pooled samples, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 25 pmol dNTPs and 0.8 U Taq DNA polymerase (Fanzyme). Thermal cycling (Perkin Elmer 9700) was carried out by initial denaturation at 94°C for 2-5 min, followed by 34-45 cycles each at 94°C for 30-60s, annealing temperature at 28-54 for 30-60s (Table 1), polymerization temperature at 72°C for 30-60s and final extension at 72°C for 10 min. The samples were cooled at 4°C. The amplified DNA fragments were separated on 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. PCR products generated from 18S rRNA gene amplification were digested with various restriction endonucleases: *BamHI, EcoRI, HindIII, SmaI and XbaI*. Where, one unit is defined as the amount of enzyme required to digest 1 µg of DNA in 1 hour at 37°C (25°C for *SmaI*) 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 three flocks. The index of similarity between each two flocks was calculated using the formula: Bab = 2 Nab/ (Na + Nb), where Nab is the number of common fragments observed in individuals a and b flocks, and Na and Nb are the total number of fragments scored in a and b respectively[19]. 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 using the average linkage between groups statistical system.

RESULTS AND DISCUSSIONS

Results: Ten random and 18S rRNA gene primers (Table 1) were used to identify the genetic similarity among the three Egyptian flocks: Beheiry, Minoufy and Saidy. All ten primers, in addition to 18S rRNA gene primer, were successfully amplified on the genomic DNA from pooled samples of each flock separately. Figure 1 shows the polymorphic bands of the first three random primers (1-3, as an example) among the three flocks under study, whereas PCR amplification of the gene encoding 18S rRNA in Beheiry, Minoufy and Saidy flocks yielded fragment of 600 bp (Figure 2).

RAPD analysis was used to construct the parsimony tree depicting relationships among the three flocks studied (Figure 3). Data presented in Table 2

Table 3: Comparison of phenotypic characters among the three Egyptian water buffaloes.

Character	Beheiry buffalo	Minoufy buffalo	Saidy buffalo
1- Skin color	Fair	Fair	Dark
2- Hairs	Heavy on the neck and the shoulders	Rare	Heavy
3- Size	Big	Medium	Small
4- Milk production	High	Medium	Low
5- Features	The organs are long and the horns are long and converted to back	The organs are compacted and the horns are small	The organs are compacted and the horns are big, open and have different directions

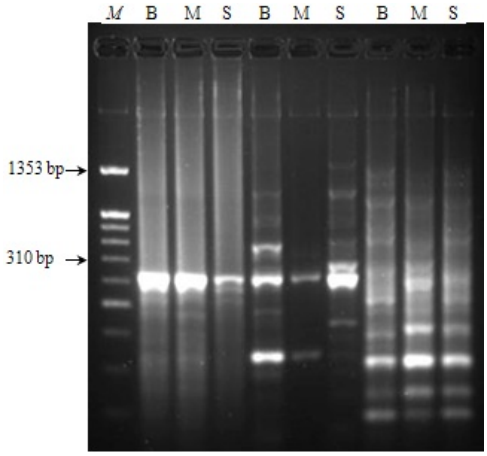

Fig. 1: An example of RAPD patterns in the three Egyptian water buffalo flocks obtained with different random primers (1-3). Lane M: ?X174 DNA marker, lane B: Beheiry, lane M: Minoufy and lane S: Saidy.

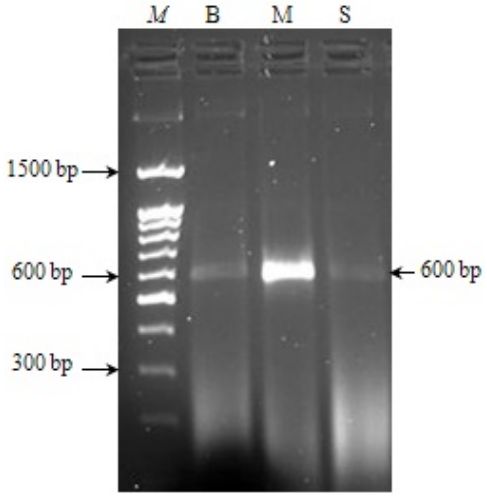

Fig. 2: Agarose gel electrophoresis of amplified 18S rRNA gene (600 bp). Lane B is Beheiry, lane M is Minoufy, lane S is Saidy and lane M is a molecular weight marker (100-bp ladder).

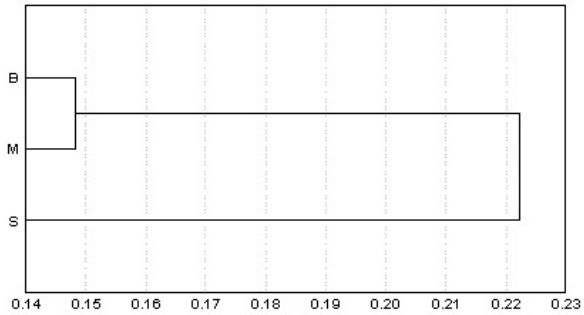

Fig. 3: Dendrogram using Average Linkage based on RAPD data analysis among the three flocks of Egyptian water buffalo. Where, B: Beheiry, M: Minoufy and S: Saidy.

showed estimated genetic similarity among Beheiry, Minoufy and Saidy flocks which was 85%, 74% and 81%, respectively.

Discussion: The Egyptian water buffaloes were phenotypically classified to three flocks according to their locations. However, Table 3 summarized the phenotypic characters of these three flocks (Beheiry, Minoufy and Saidy) and the phenotypic differences among them. In the present study, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) for 18S rRNA gene techniques were applied to construct the genetic similarity among the three Egyptian water buffalo flocks and to confirm their phenotypic classification.

As can be seen, results presented in Figure 3 and Table 2 showed three flocks of water buffalo (Beheiry, Minoufy and Saidy) in Egypt. However, the closer proximity or the highest genetic similarity was observed between Beheiry and Minoufy (85%), while the lowest genetic similarity was observed between Beheiry and Saidy (74%). These findings in this study confirmed the classification of Egyptian water buffaloes to three flocks (Beheiry, Minoufy and Saidy) based on the genetic similarity among these three flocks. On the other hand, the results of RFLP for 18S rRNA gene technique showed that, no genetic variations were

noticed among the three Egyptian water buffalo flocks: Beheiry, Minoufy and Saidy. The RAPD technique has also been used for constructing phylogenetic relationships in other farm animals such as; cattle[12,16,14,17,24,28], goat[1,5,18], horse[4] and sheep[20,6,26,15,2,21] .

In conclusion, this work has revealed that genetic diversity exists among the three Egyptian water buffalo flocks studied. With further experimentations, the RAPD profile generated for each flock 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[3,7,23].

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