ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Genetic Diversity Among horse Lines in Erbil Region Using RAPD Markers

Hurea S. Abdulrazaq [1], Chiman H. Saeed [2], Nazhad H. Qader [3]

[1] Department of Animal Resource ,College of Agriculture, Salahaddin University-Erbil / Kurdistan Region, Iraq
[2] Medical Research Center, Hawler Medical University, Erbil / Kurdistan Region, Iraq
[3] Department of Animal Resource ,College of Agriculture, Salahaddin University-Erbil / Kurdistan Region, Iraq

A B S T R A C T:

Genetic diversity is the basis for present day diversified living systems and future genetic improvement needs. Within the framework of breed conservation, genetic characterization is important in guarding breeds and is a prerequisite for managing genetic resources. The objective of this study was to use the RAPD technique to evaluate genetic diversity and relatedness within and among four horse line (white, Gray, Brown, Black). To our knowledge there is currently no information about RAPD genetic markers that detect genetic polymorphism in Erbil/Iraqi horse breeds. Information from this work provides basic genetic knowledge that is critical for conservation and breeding programs. Random amplification of polymorphic DNA (RAPD-PCR) was done by using 10 primers from GenScript USA company. A total of (6) Primers out of the (10) Primers gave results to find a complementary DNA Genomic sites, (OPQ-05, OPQ-06, OPQ-08, OPQ-09, OPQ-10, OPQ-12). These primers amplified on average 7 to 53 bands of sizes varying from 100bp to 1500bp. A total of 150 diagnostic bands were scored within RAPD profiles amplified by these 6 primers. Among 150 scorable bands 28 (18.67%) were recognized as polymorphic. UPGMA dendrogram based on Nei's genetic distance grouped the investigated horse line genotypes into two clusters. The first cluster includes(white, Brown, Black) whereas the second cluster include Gray which appeared to be most distant from the other lines. In conclusion, these results indicated the effectiveness of RAPD in detecting polymorphism between horse lines and their applicability in lines studies and establishing genetic relationships among the horse lines.

KEY WORDS: Genetic diversity, Genetic improvement, Polymorphism, RAPD markers, Horse.
DOI: http://dx.doi.org/10.21271/ZJPAS.31.3. 6
ZJPAS (2019) , 31(3);39-44 .

1. INTRODUCTION :

Horses have participated in civilization in close association with man as well as in both historical and contemporary societies. They have played major agricultural, economic and cultural roles. Horses are members of a family. In Erbil / Iraq, there are four lines of horses (white, gray, brown, black). Different horse has been developed to perform different functions.

The horses are used for racing, and others are used as high-stepping horses. Many people began to use horses in various activities such as horse sports. The development of molecular techniques has provided new possibilities for selection and genetic improvement of livestock. Research into eukaryotic genomes is influenced by the discovery of polymerase chain reaction and has contributed to the development and application of various DNA markers (Marle-Koster and Nel, 2003). The most important part of the evolutionary genetics toolkit is the molecular markers derived from the PCR reaction of genetic DNA (Holsinger et al., 2002). Meeting current production needs in different environments requires the genetic

* **Corresponding Author:**
Hurea Saber Abdulrazaq
E-mail:hurea.abdulrazaq@su.edu.krd or hurea.saber@gmail.com
Article History:
Received: 08/11/2018
Accepted: 15/04/2019
Published: 18/06/2019

diversity of farm animals to allow for sustainable genetic improvement and to facilitate rapid adaptation to changing breeding targets (Notter, 1999). Genetic markers can measure genetic diversity. These have been used to estimate the genetic diversity of species (Zhang et al., 2006). Genetic patterns to identify genetic diversity that combines the attributes of interest and can be used for the introduction of important agricultural traits (Rehman et al., 2006). Using observable morphological characteristics that require a lot of time and effort and to solve the problem of maintaining pure strains, the use of molecular markers in maintaining the horse strain is more appropriate and less time consuming. To identify and estimate genetic distances, many researchers used multiple-randomized DNA (Williams et al., 1990; Welsh and McClelland, 1990) to study genetic diversity within the lines (Apostolidis et al., 2001; El-Soudy et al. , 2005; Eroglu and Arica, 2009) and gene mapping in farm animals (genetic link maps now available for horses, Shiue et al., 1999). The RAPD technology has provided a fast and efficient screen-based polymorphism sequence of DNA in a very large number of sites. The main advantage is that no advance information about the DNA sequence is required. A wide range of potential primers that can be used gives a great technique to diagnose power. Reproductive RAPD ranges can be found by careful selection of primers, improved PCR conditions for target species RAPD markers have been successfully used to estimate the genetic correlation between different groups of cattle, sheep, goats, buffalo and chickens (Mahfouz et al., 2008; Hassan et al., 2007; 2006; Abdul Rahman and Hafez, 2007; Okumos and Kaya, 2005, respectively). RAPD has the potential to detect polymorphisms among horse populations and their applicability in population studies and the establishment of genetic relationships between groups of horses (Bailey and Lear, 1994; Shiue et al., 1999; Apostolidis, 2001 and Egito et al., 2007). The aim of this study was to use RAPD technique to assess genetic diversity and interrelationships within and between four lines of horses (white, gray, brown, black). To our knowledge, there is currently no information on the genetic markers RAPD that reveal the genetic polymorphism in Erbil / Iraqi horse breeds. Information from this work provides basic genetic knowledge that is critical to conservation and education programs.

2. MATERIALS AND METHODS

2.1. samples collection

Blood samples were collected about 3 ml of 40 horse belonging to four different lines (white, gray, brown, black). Blood samples were collected in tubes containing anticoagulant (EDTA), stored at -20 ° C until DNA extraction.

2.2. Genomic DNA extraction

Extract DNA from 300 µl of blood Using Kit Promega USA (Beutler et al., 1990). All laboratory work was carried out at Erbil Medical Research Center / Hawler Medical University.The purity of the DNA samples ranged from 1.5 to 1.9 after the DNA quality isolated by the Nano Drop® spectrometer. The samples were then diluted to 30 ng / µl for use of RAPD PCR In the Salahaddin Research Center in Erbil / University of Salahaddin Erbil.

2.3. RAPD- PCR analysis

The amplification of RAPD was done using 10 primers (Table 1) from GenScript USA. A total of (6) Primers out of (10) Primers gave results to find Genomic DNA complementary sites, (OPQ-05, OPQ-06, OPQ-08, OPQ-09, OPQ-10, OPQ-12). The final volume was 25 µl and had 30 ng of DNA polymerase chain reaction (PCR), and 10 µM of each primer. Calculation temperatures for the Tm parameter were calculated based on GC sequence composition. The PCR program included an introductory step for attenuation at 94 ° C for 5 min followed by 40 cycles with 94 ° C for 1 min for DNA transcription, as illustrated with each primer, extension at 72 ° C for 1 min and final extension at 72 ° C 5 minutes. PCR products were tested with electrophoresis on 2% agarose gel in 1X TBE buffer (Promega, USA) stained with ethidium bromide. The pattern was amplified by ultraviolet light and photographed.

2.4. statistical analysis

Data recording and statistical analysis RAPD patterns were recorded because of (1) or absence (0). The similarity index between each group was

calculated using the formula: similarity = 2nxy / nx + ny. And use, genetic distance = 1- (2nxy / nx + ny). The polymorphism of each primer was calculated on the basis of the following formula: - polymorphism = (Np / Nt) × 100, NP = # polymorphic forms of random primer Nt = total number of sample primer domains (Bowditch et al., 1993).

3. RESULTS AND DISCUSSION

The discovery of polymerase chain reaction has contributed to research in eukaryotic genomes and in the development and application of various DNA markers (Marle-Koster and Nel, 2003). Molecular markers derived from the polymerase chain reaction (PCR) of DNA are an important part of the evolutionary genetics toolkit (Holsinger et al., 2002). By detecting genetic variation, genetic markers have provided useful information in areas such as population structure, gene flow, evolution, and relationship and relationship analysis (Feral, 2002). In this study, RAPD technique was used and random primers were tested to amplify the genetic DNA of these lines. Six of them were selected for further analysis, based on the existence of replicated RAPD profiles and distinct in the horse line (Table 1).

These primers are inflated on average from 7 to 53 groups of sizes ranging from 100bp to 1500bp. This observed range of products is assumed to be due to limitations in the ability to dissolve agarose particles in low molecular weights as well as inefficient prolongation interaction under the PCR conditions described in the higher molecular weights (Bowditch et al., 1993). A total of 150 diagnostic ranges were recorded within RAPD profiles amplified by these six prefixes. The total number of ranges, the multiform range, the percentage of the polymorphism and the size of the random prefixes are shown in Table (2).

Of the 150 bands, 28 (18.67%) were recognized as polymorphisms. In another study, (Egito et al. 2007) reported that 13 of 146 primers produced 44 polymorphic bands among different breeds of horses (Pantaneiro, Arabian and Thoroughbred). (Bailey and Lear 1994), who study the original Arabian breeds, found an average of 3.6 polymorphic band / primer groups in the RAPD. (Martinez .1996), who studies three Brazilian breeds (Lavradeiro, Crioulo and Campolina), found 2.9 bands / primer, using 29 markers. (Apostolidis et al. 2001) found 10.2 bands / primer and 51 polyphonic bands in Greek horses (Thessalian, Skyros Pony, Pinia, Cretan and Andravida). (Egito et al. 2007) Using RAPD-PCR markers in Study of genetic variability between Pantaneiro, Arabian and Thoroughbred horses they found 3.38 bands / primer. Thus, the percentage of primer capable of detecting polymorphisms among the assessed strains depends on the genetic background of the breeds and the genetic distance between the genome strains and their complexity (Ahlawat et al., 2004). The present study also revealed that the OPQ-8 primer produced the maximum number of bands (53), while the minimum number of bands (7) was recorded in the OPQ-12 primer in all strains (Table 2)**.**

It has been suggested that the OPQ-8 primer sequence may occur frequently in all breeds and record the largest number of polymorphic bands while the OPQ-12 Primer has been found to have the least polymorphism between the lines. (Sharma et al. 2001) found the RAPD technique that detects sufficient polymorphism within and between strains. Current and previous studies (Wei et al., 1994; Bailey and Lear, 1994; Smith et al., 1996; Egito et al., 2007) suggest that RAPD analysis requires a large number of random primers to detect polymorphism, The amplification of arbitrary primers depends on the presence or absence of corresponding primer binding sites in the genome. Thus, relatively large numbers of random primers are needed to detect enough polymorphisms to be used for gene analysis. Figures 1, 2, 3, and 4 show amplification pattern of genomic DNA of different breeds with various random declaimer primers .

The RAPD profile generated from these prefixes was used to estimate genetic diversity and interrelationships within and among horse lines based on the band frequency. The number of bands amplified in each primer was variable between the three horse breeds (Table 3). The

maximum number of bands were found in the gray line (44) followed by black (41).

UPGMA dendrogram is based on the Nei's genetic distance grouped the investigated horse line gene investigation structures into two clusters. The first clusters (white, brown, black) includes and the second clusters include (gray) that appears to be farther than the other lines. The dendrogram, based on the genetic distance, was constructed to show the phylogenetic relationships between the horses' lines (Fig. 4). Gray seems to be far from the other lines while the lines (white, brown, black) are closely related to the highest genetic similarity. The close identity of the white, brown and black lines suggests that all the original breeds are relatively similar to the same evolutionary tree line.

4. CONCLUSIONS

The present work was designed to determine the effectiveness of RAPD markers in detecting the polymorphism and estimating the genetic relationship between horse lines. (Gray) seems to be far from the other lines while the lines (white, brown, black) are closely related to the highest genetic similarity.

Table 1. Sequence, operon codes and GC content of random primers used to study variation in horse lines.

Primer Name	Sequence 5' to 3'	%GC content
OPQ-01	GGGACGATGG	70%
OPQ-12	TCTCCGCAAC	60%
OPQ-15	GGACGCTTCA	60%
OPQ-05	GGGTAACGCC	70%
OPQ-06	CAATCGCCGT	60%
OPQ-8	CAGCACCCAC	70%
OPQ-9	CCCCGATGGT	70%
OPQ-10	ACGGACGTCA	60%
OPQ-12	AGTGCGCTGA	60%
OPQ-11	TGTGCCCGAA	60%

Table (2) Total number of bands, polymorphic band, % of Polymorphism and their Size ranges from the random primers.

Primer number	Total number of bands	polymorphic band	% of Polymorphism	Size (bp) Min.	Max.
OPQ-05	15	1	6.67	500	1000
OPQ-06	25	12	48.00	100	1400
OPQ-8	53	5	9.43	220	1200
OPQ-9	15	7	46.67	100	800
OPQ-10	35	2	5.71	200	1500
OPQ-12	7	1	14.29	400	500
	150	28	18.67		

Table (3) Number Of Band Per Primer In Different Horse Line

Primer number	White	Gray	Brown	Black
OPQ-05	4	3	4	4
OPQ-06	6	5	2	12
OPQ-8	15	15	10	13
OPQ-9	1	11	1	2
OPQ-10	10	8	8	9
OPQ-12	2	2	2	1
Total	38	44	27	41

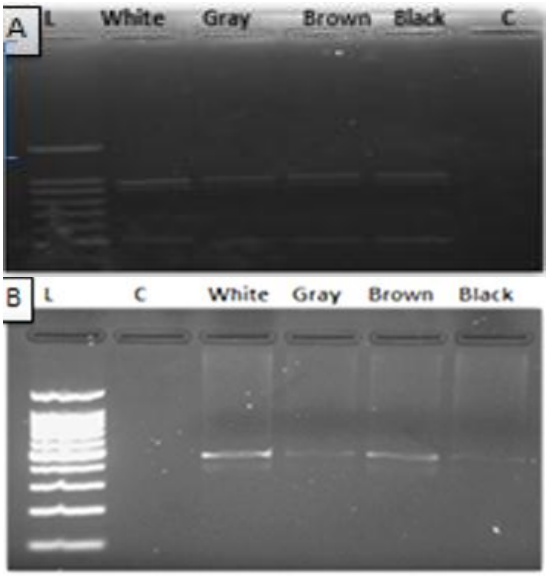

Fig (1). A. Gel electrophoresis for (OPQ-05) RAPD primer products. B. (OPQ-012) primer, for four different lines (white, Gray, Brown and Black) samples, C-Control, L - Molecular marker(100 bp plus ladder).

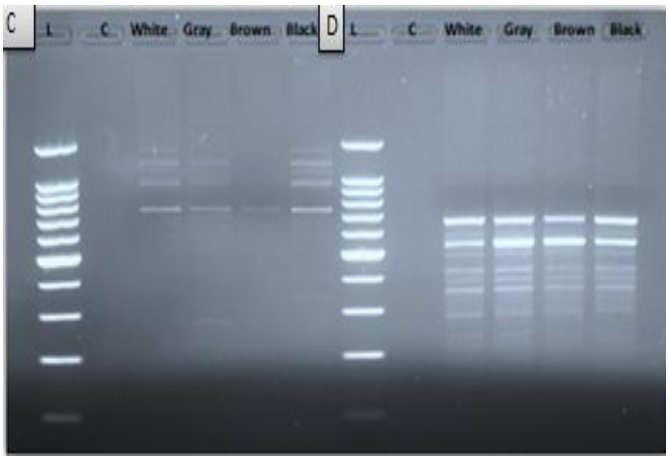

Fig (2) C. Gel electrophoresis for (OPQ-06) RAPD primer products. D.(OPQ-08) primer, four different line(white, Gray, Brown and Black) samples, C-Control, L - Molecular marker (100 bp plus ladder).

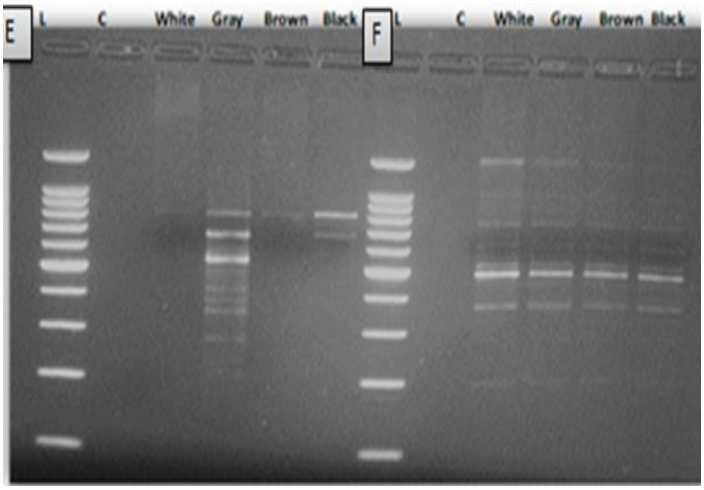

Fig (3). E. Gel electrophoresis for (OPQ-09) RAPD primer products. F. (OPQ-10). primer, four different line(white, Gray, Brown and Black) samples, C-Control, L - Molecular marker (100 bp plus ladder).

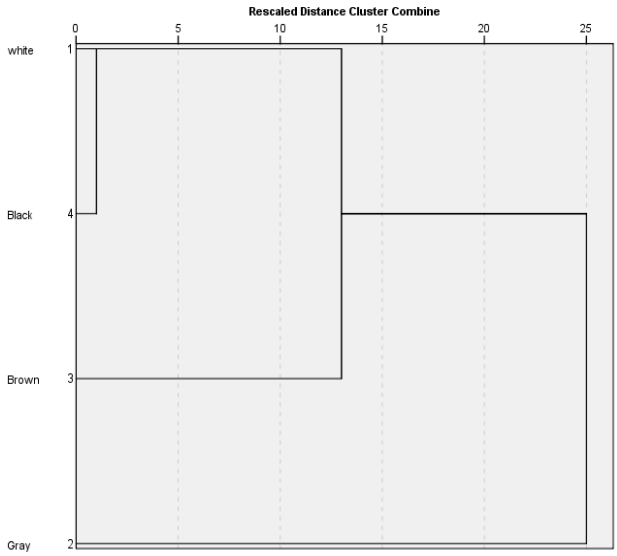

Figure (4) dendrogram of four Horse lines based on genetic distances among them.

5-REFERENCES

Abdel-Rahman, S.M. and Hafez, E.E. (2007): Genetic Similarity Among the Three Egyptian Water Buffalo Flocks Using RAPD-PCR and PCR-RFLP Techniques. Research J. Agri. and Bio. Sci. 3 (5): 351- 355.

Ahlawat SPS, Sunder JDr, Kundu A, Chatterjee R N, Rai RB, Kumar B, Senani S, Saha S K, Yadav S P (2004). Use of RAPD-PCR for genetic analysis of Nicobari fowl of Andamans. British Poultry Science. 45(4): 194 - 200.

Apostolidis AP, Mamuris Z, Karkavelia E, Alifakiotis T (2001). Comparison of Greek breeds of horses using RAPD markers. J. Animal Breed. Genet. 118: 47-56.

Apostolidis AP, Mamuris Z, Karkavelia E, Alifakiotis T (2001). Comparison of Greek breeds of horses using RAPD markers. J. Animal Breed. Genet. 118: 47-56.

Bailey E and Lear TL (1994). Comparison of Thoroughbred and Arabian horses using RAPD markers. Anim. Genet. 25 (Suppl. 1):105-108.

Beutler, E., Gelbar, T . Aand Kuhl , W . (1990). Interfrence of heparin with the polymerase chain reaction . Bio Techniques 9,166.

Bowditch BM, Albright A, Williams J, Braun MJ (1993). The use of RAPD markers in comparative genomes studies. Meth Enzymol. 224:294–309.

Egito AAdo, Fuck BH, McManus C, Paiva SR, Albuquerque Mdo SM, Santos SA, Abreu UGPde, Silva JAda, Sereno FTPdeS, Mariante AdaS (2007). Genetic variability of Pantaneiro horse using RAPD-PCR markers. R. Bras. Zootec.36 (4):799-806.

El-Seoudy AA, Abdel Gawad NM, Abu-Shady AM, Abdelsalam AZE (2005). Biochemical and molecular characterization of some Egyptian goat breeds. Egypt. J. of Genetics and Cytology. 34: 63-79.

Eroglu D, Arica ŞÇ (2009). Molecular genetic analysis of three Turkish local silkworm breeds (Bursa Beyazı, Alaca and Hatay Sarısı) by RAPD-PCR method. Journal of Applied Biological Sciences. 3(2): 15-18.

Feral, J.P., 2002. How useful is the genetic markers in attempts to understand and manage marine biodiversity. J. Exp. Mar. Biol. Ecol., 268: 121-145.

Hassen F, Bekele E, Ayalew W, Dessie T (2007). Genetic variability of five indigenous Ethiopian cattle breeds using RAPD markers. Afr. J Biotech. 6 (19): 2274-2279.

Holsinger, K.E., P.O. Lewis and D.K. Dey, 2002. A Bayesian approach to inferring population structure from dominant markers. Mol. Ecol., 11: 1157-1164.

Holsinger, K.E.; Lewis, P.O. and Dey, D.K. (2002) A Bayesian approach to inferring population structure

from dominant markers. Mol. Ecol., 11: 1157-1164.

Mahfouz, E.R.; Othman, O.E.; Soheir M.E. and El Barody, M.A.A. (2008): Genetic variation between some Egyptian sheep breeds using RAPD-PCR. Research Journal of Cell and Molecular Biology, 2 (2): 46-52.

Marle-Koster, E.V. and L.H. Nel, 2003. Genetic markers and their application in livestock breeding in south Africa: A review. S. Afr. J. Anim. Sci., 33: 1-10.

Martins VB (1996). Técnica de diagnóstico com marcadores RAPD para uso e preservação de germoplasma eqüino. Brasília: Universidade de Brasília. pp. 44.

Nei, M. (1972). Genetic distance between populations. American Naturalist, 106.

Notter DR (1999). The importance of genetic diversity in livestock populations of the future.J Anim. Sci.77:61-69.

Okumus A, Kaya M (2005).Genetic similarity by RAPD between pure lines of chickens. J. of Biol. Sci., 5(4): 424-426.

Rahman MA, Rahman MM, Jalil MA, Uddin SN, Rahman MM (2006). Molecular characterization of Black Bengal and Jamuna Pari goat breeds by RAPD Markers. American J. of Animal and Veterinary Sciences. 1 (2):17-22.

Sharma D, Appa Rao KB, Singh RV, Totey SM(2001). Genetic diversity among chicken breeds estimated through randomly amplified polymorphic DNA. Anim. Biotechnol. Nov, 12(2):111-20.

Shiue Y-L, Bickell LA, Caetanoa AR, Millon LV, Clark RS, Eggleston ML, Michelmore R , Bailey E, Guerin G, Godard S, Mickelson JR, Valberg SJ, Murray JD, Bowling AT (1999). Synteny map of the horse genome comprised of 240 microsatellite and RAPD markers. Anim. Genet. 30(1): 1-9.

Williams J.G.K.; Kubbelik A.R.; Livak K.J.; Rafalski J.A. and Tingey S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18, 6531-6535.

Zhang L, Zhu J, Gu S, Sun Q, Zhou G, Fu C, Chen L Li D, Liu S, Yang, Z (2006). Genetic diversity of nine population of black goat (Caprahircus) in Schuan, P R China. Zoolog. Sci., 23(3):229-234.