Evaluation of Genetic Variation of Local and Holstein Cattle by RAPD-PCR Technique

Article *in* International Journal of Science and Research (IJSR) · December 2017

DOI: 10.21275/ART20178710

1 author:

Asaad Yheia
University of Basrah
42 PUBLICATIONS **10** CITATIONS

SEE PROFILE

International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Evaluation of Genetic Variation of Local and Holstein Cattle by RAPD-PCR Technique

Asaad Y. Ayied[1], Majeed Sh. Al-Omiri[2]

Animal Production Department, College of Agriculture, University of Basra, Basra, Iraq

Abstract: ***The present study has been conducted in the laboratory of Molecular Genetic/ College of Agriculture/ University of Basra during 2017 to evaluate the genetic variations and similarity between local breedand Holsteinbreed using the technique of RAPD-PCR. A total of 60 blood samples were collected from Al-Qadissya governorate, which was 30 local breed samples and 30 Holsteinbreed samples. The DNAs of examined samples were extracted and used as a template for RAPD-PCR by employing five different RAPD primers which were OPC-01, OPC-04, OPC-06, OPC-07,and OPC-09.All of theused primers showed polymorphism results with an exception for the primer of OPC-04 which failed to amplify the DNAs in all examined samples. The result of allprimer showed a range of amplified band of 100-1000 bp. The total number of amplified bands in thelocal breed was 187 bands divided into185(98.4%) polymorphic bands and threemonomorphic bands. Holstein showed a total of 148 amplified bands; 144 (98.4%)polymorphic bands and fourmonomorphic bands. The lowest molecular weight of DNA products in thelocaland Holstein breeds was 100bp. Both examined breeds shared a total of 64 bands.The genetic similarity and variations between local and y breed were found to be 16.17% and 83.83% respectively; while the highest genetic similarity was observed in the primer OPC-09 which was 35.06%; the lowest genetic similarity was observed in the primer OPC-07 which was 6.15%. Generally; the genetic variations seems to be high (80%) in all employed RAPD primers. Total, within and between genetic diversitywas 46.86%, 41.02% and 12.46% witha gene flow of 3.512. These results could be attributed to the fact that the Holsteinbreedwas under continuous selectionunder different environmental conditions for long periodsof milk production; while the local breed did not expose to any type of selections until the time of thestudy.***

Keywords: Breed characterization, cattle, DNA fingerprinting, diversity, RAPD-PCR

1. Introduction

The extinction of any genotypes or local breeds may result in the loss of some available alleles. Iraqi cattle breeds are among the endangered species because there is no strategy of breeding and scientific improvement under systematic supervision. Depending on the phenotypic structure, several local breeds of cattle can be described in Iraq, the most important of which is the southern, Rosetaki and Karadi breeds (Al-Qudsi and Elia, 2014). So far there has been a severe scarcity of studies on the performance of domestic cattle or their genetic structures (genetic studies).The susceptibility to detecting the genetic polymorphisms of DNA has allowed for a new direction for genetic analysis of farm animals (Guneren and Ertugrul, 2010). In particular, understanding the pattern of genetic variability between breeds may help to develop breeding programs better accepted (Elmaci*et al*., 2007).Genetic markers are powerful tools for searching for the genetic relationship between breeds (Caetano-Anolles *et al*., 1991). There are many molecular markers that can be diagnosed for different animal species such as protein morphology, randomized amplification of DNA RAPD, RFLP, VNTR, SNP, STR, and microsatellite (Scott *et al*., 1992).The PCR-RAPD technique was developed by Williams *et al*. (1990). It relies on the serial interaction of PCR using primers similar to random areas of the genome. The use of this technique may be successful in studying population composition and genetic variability. The main advantages of this technology are its ease, low cost and fast compared to other technologies. This technique was used to determine the relationships between different breeds and individuals and to formulate breeding strategies as well as their use in controlling animal pedigree (Crowhurst*et al.*, 1991). To understand the origin of the genetic variability of livestock breeds or their populations and the relationship between these breeds may help preserve livestock sources and develop breeding programs (You *et al* 2006).

This study is important to be one of the first studies to describe one of the local Iraqi cattle breeds and compare it to the Holsteinbreed. The main purpose of this study is to investigate the use of thePCR-RAPD technique to calculate the genetic variation within the local breed and between the two studiedbreeds. As well as we expect that the results of this study may help to protect local breeds.

2. Materials and Methods

The present study has been conducted in the laboratory of Molecular Genetic/ College of Agriculture/ University of Basra during 2017 to evaluate the genetic variations and similarity between local breed and Holstein breed using the technique of RAPD-PCR. A total of 60 blood samples were collected from Al-Qadissya governorate, which was 30 local breed samples and 30 Holstein breed samples. The DNAs of examined samples were extracted and used as a template for RAPD-PCR by employing five different RAPD primers which were OPC-01, OPC-04, OPC-06, OPC-07, and OPC-09 (table, 1).

Table 1: Primers used in RAPD-PCR technique

Primer name	Symbol	Sequence 5"-3"	Annealing Temperature	
			◦C	Length (base)
1	OPC-01	TTC GAG CCAG	36	10
2	OPC-04	GAA CGG ACTC	36	10
3	OPC-06	GTC CCG ACGA	36	10
4	OPC-07	AAA GCT GCGG	36	10
5	OPC-09	TGT CAT CCCC	36	10

Blood Samples

Blood samples (5 ml/animal) were collected from all animals using a 5 ml syringe. The blood samples were placed in vacuum tubes containing ethylene diamine Tetra Acetic Acid (EDTA), and the blood samples were kept at a temperature of 4 ° C until DNA extraction was performed.

DNA extraction

DNA was extracted from the blood samples of cows using the diagnostic kit produced by Invitrogen, US according to the following steps with some necessary adjustments. RAPD-PCR amplification of each animal was performed in 13.2 µl reaction mixtures containing; 0.2 mM of primer, 1.25 U TaqTM polymerase 25,mM MgCl2, 10 mM dNTP and 200 ng of genomic DNA. Amplifications were performed using a TechneTM thermal cycler that was programmed for 45 cycles of at 94° C for 1 min, at 35° C for 30 sec and at 72° C for 1 min,and a final extension at 76°C for 6 min for elongation (Anderson et al., 1986). The amplified products were electrophoresed by running in a %1.4w/v agarose gel at 1-2 V/cm for approximately 4-6 h in the1×TAE buffer. The gels were stained with Ethidium Bromide, viewed under UV light and documented for further analysis.

Statistical analysis

Only distinct and prominent bands were scored and thepresence and absence of a band were recorded as '1' and '0',respectively. POP Gene program (Yeh *et al* 1999) was used to analyze data collected from local and Holstein breeds.

3. Results and Discussion

The ability to detect genetic polymorphism at the DNA level has led to the development of new trends in genetic analysis in different animal species. Useful PCR-RAPD technique contributes to this genetic analysis, especially in the areas of cross-species and species relations. All of the primers used in this study showed amplification by a large group of bands, some of which showed genotypic polymorphism and the other did not. Figure 1 shows the genome of the cows and electrophoresis of agarose 0.02 for the PCR product of the five primers used in this study, whereas the primer OPC-01 showed no genetic makeup. These results correspond to those obtained by Atta et al (2009) using the PCR-RAPD technique and the same primers used for Egyptian cattle.

Total, special and common bands of study profiles

The total number of bands of all the studied primers was 187 in the local breed and 147 in the Holstein breed (Table 2). The OPCO 9 showed the highest number of total bands compared to the number of bands shown by the other primers in the local breed (58 bands). The lowest bands number showed by OPCO 4 in the local breed (only 29 bands). While the primer OPCO 8 and OPCO9 had the highest number of bands (46 bands) in the Holstein breed. While OPCO 7 showed the lowest number of bands in the Holstein breed (4 bands only).

Table (2) also shows that the number of special bands shown by the primers in the local breed is 74, while the Holstein breed showed 81bands. The largest number of special bands in the local breed was shown by the OPCO 6 primer (25 bands) and the lowest bands of this breed were shown by the OPCO 7 primer (10 bands). Both breeds shared a total of 60 bands. OPC09 and OPC06 primers exhibited the highest shared bands (27 and 20 bands) and OPC07 and POC04 showed the lowest number of shared bands (4 and 9 bands only).

Table 2: Total, special and shared bands of local and Holstein breeds

Primer	Local breed		Holstein breed		Shared bands
	Total bands	Special bands	Total bands	Special bands	
OPC04	29	19	41	17	9
OPC06	45	25	46	24	20
OPC07	55	10	14	9	4
OPC09	58	20	46	31	27
Total	187	74	147	81	60

Similarity and variation between breeds

The increase in shared bands correlates negatively with variation between breeds. As a number of shared bands increases, variation between breeds decreases. Similarity and variation between the two studied breeds are shown in the table (3). Mean of similarity and variation were 16.17% and 83.83% respectively. OPC09 showed the highest similarity percent between the two breeds (35.06%), while OPC07 showed the lowest (6.15%) which was translated into the highest variation (93.75%) between the two breeds. Generally, variation between the two breeds was very high (more than80%) for all primers. This might be originated from high genetic variation between the two reed, as Holstein was heavily selected for very long time for high milk yield, while local breed lack any type of systematic selection. As well as these two breeds initiated under very divergent environments.

Table 3: Similarity % and variation between local and Holstein breeds

Primer	Similarity %	Variation %
OPC04	14.75	85.25
OPC06	19.79	80.28
OPC07	6.15	93.85
OPC09	35.06	64.93
MEAN	16.17	83.83

Similarity%= number of shared bands*100/total bands, Variation%=different bands*100/total bands

Similarity and variation within breed

Table (4) summarized similarity and variation percentages within each breed. From the results of thetable (4), both breeds showed a similarity higher than 70% (local breed: 78.34> Holstein 72.37%), which reflected the non-systematic breeding of local breed and indicate some kind of inbreeding within this breed more than Holstein breed. Variability demonstrated by different primer ranged between 7.94 (primer OPC09) to 28.89% (primer OPC06) for thelocal breed. That of Holstein was 15.56% (OPC06) to 53.85% (primer OPC04).

Table 4: Similarity and variability within Local and Holstein breeds

Primers	Local breed (%)		Holstein breed (%)	
	Similarity	Variability	Similarity	Variability
OPC04	**73.71**	**26.83**	**46.15**	**53.85**
OPC06	**71.11**	**28.89**	**48.44**	**15.56**
OPC07	**72.73**	**27.27**	**76.47**	**23.53**
OPC09	**92.06**	**7.94**	**55.32**	**44.68**
Mean	78.4	**21.57**	**72.37**	**27.63**

Polymorphisms

Table (5) indicates polymorphisms of both studied breeds for every four primers, as well as the mean of polymorphisms of each breed. Local breed showed a polymorphism of a range of 96.55%-100%. Holstein breeds exhibited alower range of 92.86%-97.83%. Primer OPC09 revealed the highest polymorphism in both breeds. On contrary prime OPC04 showed the lowest polymorphism in local breed and primer OPC07 in Holstein.

Table 5: Number of single and polymorphism bands and polymorphism% of local and Holstein breeds

Breed	Bands	Primers				Total
		OPC04	OPC06	OPC07	OPC09	
Local	Single	1	1	1	0	3
	Polymorphism	28	44	55	58	185
	Polymorphism%	96.55	97.78	98.18	100	98.40
Holstein	Single	1	1	1	1	4
	Polymorphism	41	45	13	45	144
	Polymorphism%	97.62	97.83	92.86	97.83	97.30

Primer OPC04 and OPC09 showed the highest genetic diversity (50.00% and 48.10% respectively) in local and Holstein breeds respectively (table, 6). While the primer OPC07 showed the lowest in both breeds (26.04% and 33.80% for local and Holstein breed respectively).In general Holstein breed revealed higher genetic diversity than local breed (42.14% and 39.9% respectively). When data of both breeds were analyzed together a range of genetic diversity was 43.85% for theOPC09 primer to 49.81% for OPC07 with an average of 46.85%.

Local breed characterized by higher Shannon index (69.31%) by primer OPC04 and lowest index (42.93%) by primer OPC07 with an average of 58.45% (table, 6). Holstein breed recorded thehighest mean of Shannon index (61.11%), ranged from 52.10% by primer OPC07 to 67.41% by primer OPC09. Both breeds showed an average of 66.10%, ranged from 63.33 to 69.13% by the primer OPC09 and OPC07 respectively.

Table 6: Genetic diversity% and Shannon index for information of local and Holstein breeds

Primer	Local breed		Holstein breed		Both breeds	
	H	I	h	I	Local	Holstein
OPC04	55.00	69.31	41.02	60.05	47.72	67.01
OPC06	46.42	65.69	45.63	64.87	46.03	65.29
OPC07	26.04	42.93	33.80	52.10	49.81	69.13
OPC09	37.17	55.87	48.10	67.41	43.85	63.03
Mean	39.91	58.45	42.14	61.11	46.85	66.12

***h=genetic diversity (Nei, 1973), I= Shannon index (Lewontin, 1972)**

Genetic diversity analysis

Analysis of genetic diversity a set of measurements included total genetic diversity and for each breed, as well as inbreeding coefficient and gene flow (table, 7). Total diversity ranged 43.85-49.81% with an average of 46.86%. Primer OPC07 showed the highest diversity (49.81%), while the primer OPC09 showed the lowest (43.85%). Diversity within breed ranged from 29.92% to 46.02% recorded by primer OPC07 and OPC06 respectively. The mean diversity of all primers within each breed was 41.02%.

The value of gene differences between the two breeds associated negatively with total or within genetic diversity (table, 7). Primer OPC06 revealed thetotal and within-breed diversity of 46.03% and 46.02% respectively but its gene differences coefficient was the lowest (0.0002). whereas, the primer that showed lowest genetic diversity especially within the breed (OPC07) recorded highest gene variation coefficient (0.3994). the mean of gene variation coefficient of all primers was 0.1246.

Gene flow represents a number of shared bands between the two breeds. It is calculated from gene variation coefficient. It's value increased as gene variation coefficient decreased. Primer OPC06 showed the highest gene flow level. The lowest gene flow level was shown by the primer OPC07 (0.751). the mean gene flow of all primers was 3.512.

4. Discussion

It is well documented that polymorphism between individuals results from sequence differences in one or both of the primer binding sites and is visible as the presence or absence of a particular RAPD band. Genetic diversity or similarity can be measured through genetic markers. These have been used to determine evolutionary relationships within and between species, genera or higher taxonomic categories (Cornuet et al., 1999). RAPD markers, generated by the Polymerase Chain Reaction (PCR) have been widely used since the 1990's.

Lower estimates of genetic similarities indicated a high degree of genetic diversity between these two studied cattle breeds. This may be due to their diverse origin and different purpose of raising these cattle. Sharma et al. (2009) calculated between breed band sharing (BS) was 70% to 93% in the Malvi breed and 68% to 88% in Sahiwal breed, found less genetic differences between breeds as like the present experiment (similarity of 16.17% and variation of 83.83%).

The present results revealed that there was a high level of genetic variation among the studied cattle breeds as indicated by the proportion of polymorphic loci (>97%). The values of pair-wise genetic distance among 20 cattle genotypes were computed from combined data sets three RAPD primers ranging from 0.834 to 0.031 (Khutan et al, 2012). Choy et al (2001) identified population-specific DNA polymorphisms to detect *Bosindicus*and *Bostaurus*in West Africa.

This study demonstrated that RAPD-PCR technique can be used to successfully differentiate cattle by variation of its

fingerprints in breed-specific DNA pools and subsequently can be used to produce noticeable fingerprints in individual animals. The polymorphism of the experimental cattle was more than 97% and suggests that the gene segregation rate has recently increased in these animals. The genetic variation in the experimental cattle populations was high and therefore, there is the scope of selective breeding for future improvement of local cattle. Gradual genetic erosion and extinction are threatening an increasing number of animal species; the major consequence being a loss of global genetic diversity. Sets of genetic markers are required to identify distinct populations so that they can be preserved. The local Iraqi cattle is of particular importance because of their favorable meat and milk although their productivity is relatively low in general. Like other developing countries, breed substitution and crossbreeding programs with exotic breeds have been practiced indiscriminately in Iraq for many years and as a result, valuable genetic resources are being lost or diluted. In addition, genetic assessment is also of interest for the design of genetic improvement programs including appropriate choice of breeds for crossbreeding. Thus, it is important to identify molecular characteristics of these breed/populations for undertaking proper breeding programmes and conservation strategy.

References

[1] Al-Qudsi, N. H. and Elia, J. V. (2014) Dairy Cattle Production. Animal Production Department, College of Agriculture, University of Baghdad, Iraq (In Arabic).
[2] Anderson, L., J. Bohme, L. Rask and P. A. Peterson. (1986).Genomic Hybridization of bovine class II major histocompatibility genes: 1. Extensive polymorphism of DQα and DQβ genes. Anim. Genet. 17:95-112.
[3] Atta,A.H.,Ahmed, E.S.,Sadek,M.H.andAmin, A.A.(2009). Development ofmolecularmarkers for detecting geneticrelationship withinandamong sixEgyptian buffalolocations.GlobalVeterinaria, 3(4),341-347.
[4] Caetano-Anolles G., Bassam B. J. and GresshoffP. M. (1991).DNA amplification fingerprinting using very short arbitraryoligonucleotide primers.Biotechnology,9: 553-557.
[5] Choy, Y. H., Oh, S. J. and Kang, J. O. (2001). Application of RAPD methods in meat for beef breed identification. Asian-Aust. J. Anim. Sci. 14:1655-1658.
[6] Cornuet, J. M., Piry, S., Luikart, G., Estoup, A. and Sloignac, M. (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153: 1989-2000.
[7] Crowhurst R. N., Hawthrone B. T.,Rikkerink E. H. and Templeten M.D. (1991). Differentiation ofFusarium solanif. Sp. cucurbitae race1 and 2 by random amplification ofpolymorphic DNA. Current Genet.20, 391-396.
[8] Elmaci C, Oner Y, Ozis S, Tuncel E (2007): RAPDanalysis of DNA polymorphism in Turkish sheep breeds.Biochem Genet, **45,** 691–696.Guneren and Ertugrul, 2010
[9] Khutan, M. M. Hossain, K. M. and Rahman, S. M. M. (2012). Molecular characterization of selected local and exotic cattle using cattle using RAPD marker. Asian-Aust. J. Anim. Sci. 25:751-757
[10] Lewontin R.C. (1972). Testing the theory of natural selection. Nature,236: 181-182.
[11] Nei, M. (1973). Analysisofgenediversityinsubdividedpopulations**.** ProcNatlAcad.Sci.USA**,**70: 3321-3323.
[12] Scott M. P., Haymes K. M. andWilliams S. M. (1992). Parentageanalysis using RAPD-PCR. NucleicAcids Res. 20(20), 5493.
[13] Sharma, M., Singh, S., Kushwah, A. and Sarkhel, B. C. (2009). Molecular characterization of Malvi and Sahiwal breed of cattle by RAPD-PCR. Indian J. Anim. Sci. 79:44-46.
[14] Williams, J. G. K., Kubelik, 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. Acids Res. 18:6531-6535.
[15] Yeh,F.G, Yang,R.C. and Boyle, T.(1999). POP Gene Version 1.32:Microsoft Window-Based Free Ware for Population Genetics Analysis.University of Alberta,Edmonton.
[16] You, A., Lu, X., Jin, H., Ren, X., Liu, K., Yang, G., Yang, H., Zhu, L., and He, G. (2006)**.** Identification of quantitative trait loci across recombinant inbred lines and testcross populations for traits of agronomic importance in rice. Genetics, **172**: 1287-1300.