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Genetic Polymorphism and Diversity of Iraqi native cattle (Misan province) Using PCR-RAPD

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Abstract

This study was conducted in the genetic engineering laboratory/ biology department/ college of science/Misan University.
Three primers (OPA-16, OPB-08, OPF-05) were applied in this study, all of which showed polymorphism, the highest polymorphism was with the primer OPA-16 which reached to 85% while the primer OPF-05 showed the lowest polymorphism that reached to 80%. Also the results showed that the total number of polymorphic bands was 339 with the three primers, the percentage of polymorphism was 82% The molecular weight of the bands ranged between 50-1350 bp. The results of this study explained the adaptation of local Iraqi cows with the hard climatic condition in the region as well as increase resistance to diseases .

1.Introduction

Livestock animals especially in developing countries including Iraq possesses genetic polymorphism due to adaptation with the hard climate although it led to a relatively low levels of production, therefore, it is very important to do every effort to preserve the genotypes of our native animals (Sikka, 2004). Using molecular markers in the selection programs helped in choosing animals with wanted traits, the DNA dependent markers helped in the selection of high quality animal within the same breed (Kumare, 2004). The discovery of genetic variations on the level of DNA can make revolution in the selection programs and the currently available DNA techniques can cover all the necessary requirements for this purpose, then these variants can be named (genetic markers)and selection programs built according to these markers are more reliable and accurate than any other way of selection, these markers link between physiological and biochemical processes and genetic composition(Riaz et al., 2008). There is a relatively few information available about the genetic composition of local animals in Iraq especially cows , but the PCR-RAPD technique provide the ability for better understanding of genetic relationship among breeds (Cavalier et al., 1985), this technique was rapidly applied in the selection programs (Rao et al.,1996).

2.Materials and methods

2.1.Blood samples

This study was conducted in the Genetics Engineering Laboratory of the Department of Life Sciences in the Faculty of Science, University of Misan Iraq, Blood sample used for DNA isolation was collected from 40 animals native cattle Misan Iraq. Animals were sampled from their native breeding areas based on the phenotypic characteristics of each breed.

2.2.DNA isolation and RAPD primers:

Total DNA was extracted from peripheral blood sample using salting-out procedure (Miller et al.,1988). Promega blood kit was also used to isolate genomic DNA from blood sera. three random primers (Promega, USA) Were used for DNA amplification. Each random primer was a 10-mer with GC content varying from 60% to 70%. Three primers (OPA16, OPB08, OPF05) were selected for further use in genotyping. The base sequence and length of the primers were shown in **Table 1.**

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2.3.RAPD-PCR analysis:

RAPD-PCR was carried out on DNA from individual cattle as well as pooled DNA samples (mixture of individual DNA samples within the same breed) from each breed. Breed–specific genomic DNA samples were prepared by pooling the some amount of genomic DNA from each individual of the respected breed. RAPD-PCR amplifications of each animal were performed in 13.2 µl reaction mixtures containing; 0.2 mM of primer, 1.25 U TaqTM polymerase, 25 mM $MgCl_2$, 10 mM dNTP and 200 ng of genomic DNA. Amplifications were performed using a Eppendorf thermal cycler that was programmed for 45 cycles at 94^{o}C for 1 min, at 35^{o}C for 30 sec and at 72^{o}C for 1 min, and a final extension at 76^{o}C for 6 min for elongation. RAPD-PCR amplifications of each animal were performed at least twice for confirmation of the accuracy and the repeatability of the products. Amplification products were separated by agarose gel (1.4%) electrophoresis and detected by ethidium bromide staining.

Table 1. DNA bands amplified and polymorphism genotypes using 3 RAPD markers.

Name pr	Sequence	GC %	Total Bands	Polymorphism % of	Polymorphic bands (Na.of)	Size rang (bp)	
						Minimum	Maximum
OPA-16	AGCCAGCGAA	60	193	85	164	50	1350
OPB-08	GTCCACACGG	70	116	79	92	100	1350
OPF-05	CCGAATTCCC	60	103	80	83	50	916
TOTAL			412	244	339	200	3615

3.Results

The results showed that all markers (OPF-05, OPB-08, OPA-16) has amplified genome DNA of Iraqi cows studied (**figure1**) . As evidenced by the results that have been obtained that all the markers covered by the study showed high polymorphism reached to 85% with the exception of the primer (OPB-08) has shown polymorphism79% And thus can be considered lower than other markers studied. The results of the electrophoresis of the PCR products showed that a total of 412 RAPD bands were obtained from the cattle breeds. Amplified products
ranged from 50 bp to 1350 bp in size, The maximum number of bands was obtained with the primer OPA-16 (193).

4.Discussion and Conclusion

The aim of cattle breeding is to get a high efficiency and profit per animal, therefore, high efficiency breeds have been widely preferred and raised by farmers. Due to their low efficiencies, many local breeds around the world are now under the threat of extinction. This is also the case with the Iraqi local cattle breeds, whose numbers have decreased almost by half over the last decade . Extinction of any local breed or population may result in complete loss of some valuable alleles or genetic variations which would affect the future genetic development. In spite of commercial value of Iraqi local cattle breeds, except the information on the blood groups and blood protein polymorphism, and traditional phenotypic characteristics, the population structure of these breeds are virtually unknown. Although 10 bp primers have been widely used in many RAPD-PCR studies (Ahlawat et al., 2004; Elmaci et al., 2007; Singh & Sharma, 2002; Semyenova et al., 2002). There are also reports using longer primers than 10 bp primers in cattle (Abdel-Rahman & Hafez, 2007; Ali, 2003; Alves, 2005; Loftus,1999). The results also showed variance in the number of bands for each primer ranged between 193 in OPA-16 to 103 in OPF -05 , higher number of RAPD bands would yield more reliable information about the genotypes of populations. This opinion agree with Güven G. et al.,2010.

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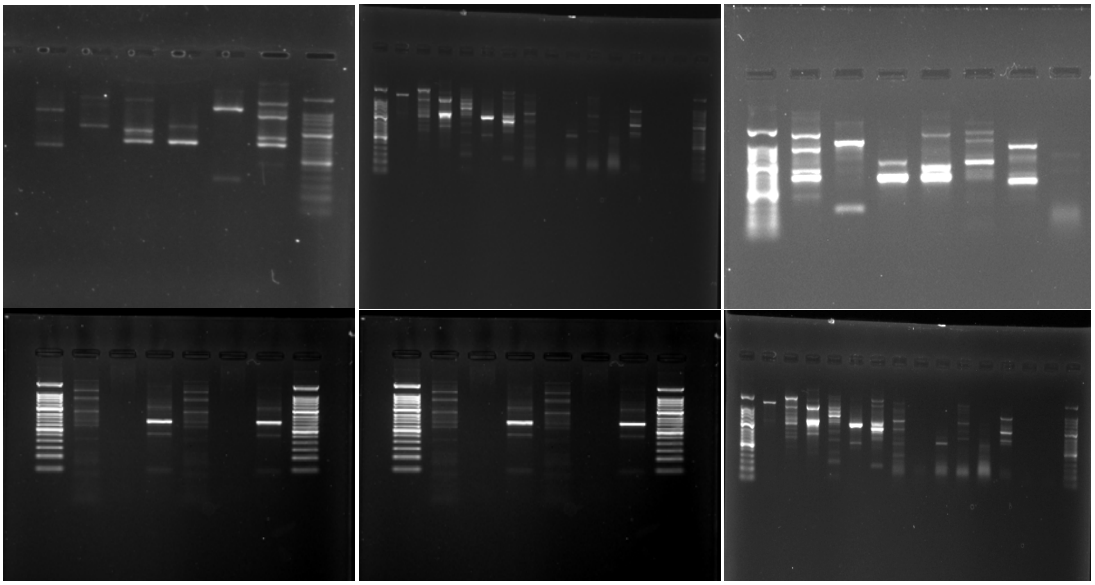

Figure 1 : Agarose gel electrophoresis of PCR products using PCR-RAPD technique.

5.References

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