

GENETIC POLYMORPHISM AND DIVERSITY OF IRAQI AWASSI SHEEP USING PCR-RAPD TECHNIQUE

Zainab S. Al-Allak *, Maytham A. Dragh **, Ahmed Sadoon Hussain *

* Department of Animal Production, College of Agriculture, University of Misan, Misan, Iraq.

** Department of Biology, College of Science, University of Misan, Misan, Iraq.

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Corresponding Author : zainabsabeeh76@gmail.com

ABSTRACT

The establishment of modern sheep production systems in Iraq, lead to presence of various forms of hybridization between the native and Middle East breeds which have been utilized for genetic improvement. This occur in consistence with the progressive destruction or deterioration of sheep habitat. Together, these factors have accelerated the loss of genetic diversity or even resulted in the extinction of some indigenous breeds. Therefore, it is important to develop efficient strategies for surveillance, evaluation, conservation and utilization of the available genetic resources for this species. Seven random amplification polymorphism DNA (RAPD) marker used. The aim of this study was to assess genetic diversity for Awassi native breed in Iraq. The higher polymorphism information contents at the seven markers (Seventy- three bands obtained with 28.3% of polymorphism) indicate the retention of natural variation from source populations for the domestic breeds of different geographic regions in Iraq. Analysis of genetic differentiation revealed substantial divergence among these breeds as 16% diversity indicating that some evolutionary forces (e.g. selection and migration, uncontrolled selling across borders) had acted on these populations. Phylogenetic and phylogeographic analyses displayed a remarkable degree of consistency between geographic origins, breeding histories and the pattern of genetic differentiation.

INTRODUCTION

Sheep was perhaps the first ruminants domesticated around 10,000 B.C. Sheep were first domesticated probably in Iran and Baluchistan Awassi is the most widespread breed of sheep from a non-European origin. It is the most common breed in the east of Mediterranean. It is the main sheep breed in Iraq and Syria while the only native breed in Jordan ⁽¹⁾. This breed adapts to a wide range of breeding systems ranging from the steppe to the highly intensive system. Performance of this breed varies according to environmental condition and strain. Iraqi strain considered the heaviest and highest milk production among all Awassi populations. Efforts to improve milk production and body weight genetically lead to positive results. In Syria, a selection program succeeded to increase weight of sheep from 128 kg in 1974–1976 to 335 kg in 2005. In Turkey, the mean milk yield of ewes increased from 67 kg to 152 kg in a selection/outcrossing program that lasted for seven years. Although Awassi best known for its high milk production, this breed is often used as a triple purpose sheep in most of the countries of its origin in the Middle East ⁽²⁾. Most of the domesticated sheep existed in Harappa and Mohenjo-Daro have traces from their origin of Mediterranean as well as Asian wild sheep. The need for conservation comes from the potential rate of decrease of genetic variation. The loss of genetic variation within and between breeds is detrimental. Once animal genetic diversity has been lost, it cannot be replaced. Indigenous and locally developed sheep breeds are an important asset for many reasons, particularly because, over time, they have developed unique adaptive traits to respond to pressures of local environment ⁽³⁾. Conservation of genetic resources requires the characterization of the available stock for preservation and management as well as evaluation of the phylogenetic origins of the genetic groups available ⁽⁴⁾. The random amplification polymorphism DNA (RAPD) marker has been widely used, due to its easy utilization by simple PCR, followed by a denaturing gel electrophoresis for number of fragments and fragments size determination ⁽⁵⁾. For all the above reasons the aim of this study was to assess genetic diversity for Awassi native breed in Iraq.

MATERIALS AND METHODS

This study conducted in the Genetics Engineering Laboratory of the Department of Life Sciences, Faculty of Science as well as in the physiology laboratory of the department of animal production, College of Agriculture, Misan University, Iraq.

Blood samples collection: Blood sample used for DNA isolation collected from 25 native Awassi Sheep in Misan province, Iraq. Animals selected for sampling based on their phenotypic characteristics of each strain from their native breeding areas.

DNA isolation and RAPD primers: Total DNA was extracted from blood sample using salting-out procedure according to ⁽⁶⁾ Promega blood kit was used to isolate genomic DNA from blood. Seven random primers (QIGENE China) used for DNA amplification. Each random primer was a 10-mer with GC content varying from 60% to 70%. Primers (OPAA11, OPU15, OPAA17, OPD18, OPA7, OPA9, and OPA10) selected for this study. The base sequence and length of the primers shown in Table 1.

Table 1: list of primers, their nucleotide sequence, range of band products, number of common and polymorphic bands, the percentage of polymorphism and diversity produced by different RAPD primers.

primer	Nucleotide Sequence 5-3	Size rang (bp)	Total Bands	Poly-morphic bands	Mono-morphic bands	% polymorphism	% Diversity
OPAA11	ACCCGACCTG	280-1100	9	3	1	% 33	11.1
OPU15	GAGAGCCAAC	200-1000	13	4	1	% 30.7	7.6
OPAA17	GAGCCCGACT	200-1450	16	5	3	% 31.2	18.7
OPD18	ACGGGCCAGT	250-1500	5	1	0	% 20	0
OPA7	GAAACGGGTG	100-1550	12	2	5	% 16.6	41.6
OPA9	GGGTAACGCC	220-800	9	2	1	% 22.2	11.1
OPA10	GTGATCGCAG	200-900	9	4	2	% 44.4	22.2
Total			73	21	13	% 28.3	16

RAPD-PCR analysis:

RAPD-PCR carried out on DNA from individual Awassi Sheep as well as pooled DNA samples (mixture of individual DNA samples within the same strain) from each strain. Strain-specific genomic DNA samples were prepared by pooling the same amount of genomic DNA from each individual of the respected strain. RAPD-PCR amplifications of each animal 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 performed using an Eppendorf thermal cycler that programmed for 45 cycles at 94° C for 1 min, at 35°C for 30 sec and at 72 °C for 1 min, and a final extension at 72°C for 6 min for elongation.

RAPD-PCR amplifications of each animal performed at least twice for confirmation of the accuracy and the repeatability of the products. Amplification products separated by agarose gel (1%) electrophoresis and detected by ethidium bromide staining.

RESULTS

The results of the DNA amplification showed that all primers (OPAA11, OPU15, OPAA17, OPD18, OPA7, OPA9, and OPA10) has amplified genome DNA of Awassi Sheep (figure1).

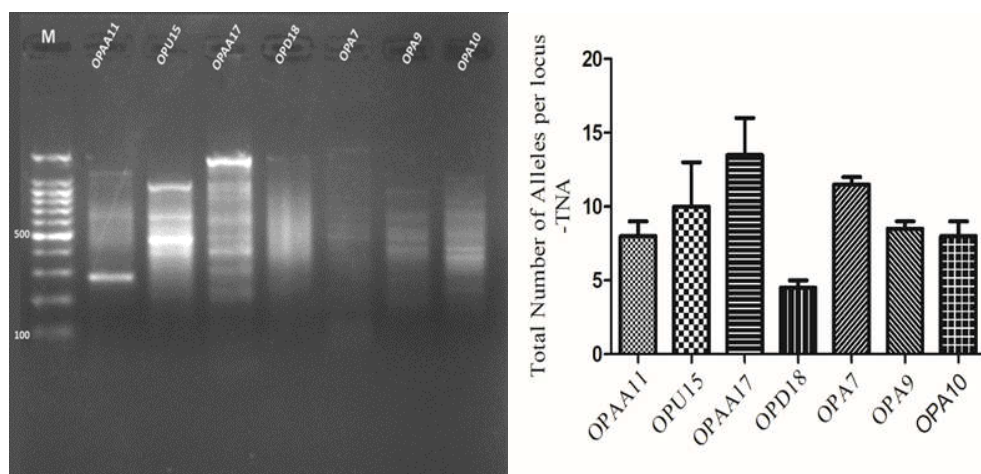


Figure 1: Agarose gel electrophoresis of PCR products using PCR-RAPD technique

In addition to, diagram (1) showed that all the primers had a high polymorphism reached to total of 28% with the exception of the primer (OPA7) has shown polymorphism 16%. The results of the electrophoresis of the PCR products showed that 73 RAPD bands obtained from the Awassi Sheep breeds. Amplified products ranged from 100 bp to 1550 bp in size, the maximum number of bands was obtained with the primer OPAA17 (16 band).

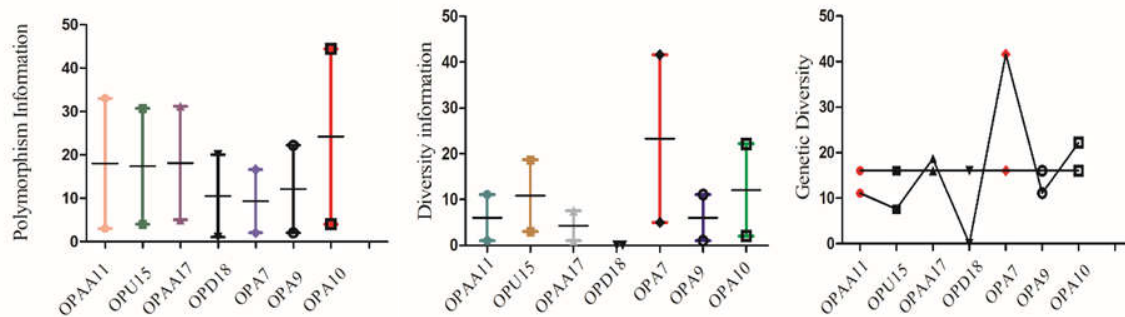


Diagram1: significance of polymorphism and diversity information and genetic diversity

DISCUSSION

High efficiency breeds widely preferred and raised by farmers as Awassi breeding. Due to low efficiencies, many local breeds around the world are now under the threat of extinction. This is also the case with the Iraqi local sheep 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 sheep breeds, the information on the blood groups, blood protein polymorphism, traditional phenotypic characteristics, and the population structure of these breeds are virtually unknown. Although 10 bp primers or longer have been widely used in many RAPD-PCR studies in livestock ^(7,8) there are also reports using longer primers than 10 bp primers in livestock ^(9,10,11). The results showed variance in the number of bands for each primer ranged between five in OPD18 to sixteen in OPAA17. Therefore, higher number of RAPD bands obtained yield more reliable information about the genotypes of populations.

Author Contributions:

Conceived and designed the experiments: ZSH and MAD. Performed the experiments: ZSH, MAD and ASH. Analyzed the data: ZSH, MAD and ASH. Contributed reagents/materials/analysis tools: ZSH and MAD. Wrote the paper: ZSH and MAD.

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عنوان البحث بالعربي(التشكل والتنوع الوراثي في اغنام العواسي العراقية باستخدام تقنية-PCR RAPD)

زينب صبيح حسن العلاق, * ميثم عبد الكاظم دراغ **, احمد سعدون حسين*

قسم الانتاج الحيواني،كلية الزراعة،جامعة ميسان، ميسان،العراق.

قسم علوم الحياة ، كلية العلوم ،جامعة ميسان ، ميسان ،العراق.

الخلاصة

ان انشاء أنظمة تربية الأغنام الحديثة أدى الى وجود اشكال مختلفة من التهجين بين السلالات المحلية وسلالات الشرق الأوسط والتي خضعت للتحسين الوراثي وهذا حدث بالتزامن مع التحوير والتدمير المستمر لبيئتها الطبيعية وبالتالي فان هذه العوامل أدت الى فقدان التنوع الوراثي ولذلك أصبح من الضروري تطوير استراتيجيات كفوة للمحافظة على المصادر الوراثية لهذه الحيوانات. استعمل في هذه الدراسة سبعة بادئات للتضخيم العشوائي للحامض النووي منقوص الاوكسجين -PCR RAPD markers. الهدف من هذه الدراسة كان تقييم التنوع الوراثي لسلالة العواسي المحلية العراقية وظهرت نتائج هذه الدراسة ان هنالك تنوعا وراثيا وصل الى ٢٨.٣% (أي ما يعادل ٧٣ حزمة تضخيم) وهذا يثبت تراجع التباين الطبيعي للمناطق الجغرافية المختلفة في العراق. ان تحليل التباين الوراثي اظهر اختلافا ثابتا ومتشعبا" بين هذه السلالات وصل الى ١٦% مما يعني ان بعض القوى مثل الانتخاب والهجرة والبيع غير المسيطر عليه عبر الحدود اثرت كثيرا" على هذه السلالات ان التحليل الوراثي والجغرافي اظهر درجة عالية من التباين بين سلالات العواسي المحلية.

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