eJBio

Electronic Journal of Biology, 2019, Vol.15(2): 062-67

Genetic Variation amongst Four Rabbit Populations I Nigeria Using Microsatellite Marker

Adewunmi O Omotoso[1]*, Olajide Olowofeso[1], Matthew Wheto[1], Olajide M. Sogunle[2]

1Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria.
2Department of Animal Production and Health, Federal University of Agriculture, PM, Abeokuta, Nigeria.

***Corresponding author:** Tel: +2348063178182; E-mail: adewunmi.omotoso@outlook.com

Citation: Omotoso AO, Olowofeso O, Wheto M, et al. Genetic Variation Amongst Four Rabbit Populations I Nigeria using Microsatellite Marker. Electronic J Biol, 15:2

Received: April 01, 2018; **Accepted:** June 17, 2019; **Published:** June 24, 2019

Research Article

Abstract

Seven microsatellite markers were used with 100 genomic deoxyribonucleic acid (DNA) isolated from New Zealand White, New Zealand Red, Californian White and Chinchilla rabbit breeds in Nigeria to determine genetic variation amongst the breeds. Power of microsatellite markers i.e., Combined Exclusion Probabilities (CEP) and Polymorphism Information Content (PIC) of markers; were determined to ascertain the informativeness of the markers. Equal number of samples (that is, 25 samples) was obtained from each of the rabbit population. Polymerase chain reaction (PCR) was carried out using each marker, isolated DNA, and double distilled water and PCR Master Mix. PCR products generated were subjected to polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. DNA bands were scored based on size of ladder with Gene Scan 3.1.2. Bands obtained were designated as alleles and prepared into Excel Worksheet. With Microsatellite Analyser version 4.05 software and allele frequencies were generated. Mean inbreeding coefficient across loci (FIS) was -0.0201 and average genetic differentiation (FST) among breeds was 0.0479. Seventy nine alleles of which 18.98% were rare and 81.02% represent fixed alleles across the rabbit populations were observed. PIC per marker across populations ranged from 0.6800 (SAT 8) to 0.8100 (SOL 28) indicating that the markers were informative (PIC ≥ 0.50). CEP across markers and populations was 0.999999, meaning that the selected microsatellite markers were suitable for parentage verification of these four rabbit breeds.

Keywords: Genetic differentiation; Informativeness; Microsatellite markers; DNA Electrophoresis; Rabbits.

1. Introduction

Rabbits described as micro-livestock specie by are ubiquitous, providing protein, fibre, experimental models and companionship [1]. Rabbit reproductive potentials and other economically important traits in it have been reiterated by Irlbeck [2]. Genetic variation has been defined as the variation within and among breeds of a given species. It is influenced by interaction of different forces such as selection, genetic drift, mutation, and migration [3] Genetic variation allows breeders to develop new characteristics in response to changes in environmental and diseases outbreak [4]. Lukefahr noted that a high degree of heterozygosity in the rabbit populations might be important for fitness-related characteristics such as fertility and survival which contributes to the final local adaptation [5].

Microsatellites, otherwise known as Simple Sequence Repeats (SSRs), are repeating sequences of 1-6 base pairs of DNA, which have been used to evaluate genetic variation and relationship in various organisms. The advantages of the marker include its ability to detect polymorphisms in many loci, higher heterozygosity, relative ease of scoring and the co-dominant nature of generated markers [6-8]. Population history of rabbit breeds kept in Nigeria till date is not available; thus, rabbit rearing could be speculated to have commenced in Nigeria at the advent of slave-trading and European invasion into Africa, when most exotic agriculturally important crops and animals were introduced [9].

Rabbit production in Nigeria is relatively at low level and the genetic characterization of the rabbit population has not yet been adequately carried out unlike the other animal genetic resources, Hence, it is imperative that genetic variation parameters (heterozygosity, mean number of alleles, F-statistics,

gene flow) be known in the populations and the exclusion probability, combined exclusion probability and polymorphism information content of the microsatellite markers that can be used with rabbit population be documented. This would facilitate long time breeding strategies, formulation of conservation policies, provide ample genetic information and will ultimately lead to rapid improvement of this important genetic stock, thereby contribute some percentages of animal protein needs of man. The objectives of the study were to determine the genetic variations among the four rabbit populations in Nigeria and the effectiveness of the microsatellite markers.

2. Materials and Methods

2.1 Blood sampling and DNA extraction

A total of hundred blood samples were collected from four rabbit breeds (New Zealand White, Californian White, New Zealand Red and Chinchilla). Equal numbers of samples (25 samples) from each of the rabbit population were collected from reputable farms and Research Institutes across Nigeria for the study. All procedures followed were done in strict adherence in accordance to the institutional animal welfare ethics.

Approximately 1 ml of blood was collected from each rabbit through the Saphenous rear leg venial puncture, aseptically into ethylene di-amine-tetracetic acid (EDTA) tubes using 1 ml sterilized syringe into 5 ml EDTA tubes, which were placed in an ice-box and later transported to the Biotechnology Laboratory, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, where the samples were stored at -20°C before the genetic material was isolated from the samples collected. DNA isolation was carried out using Norgen DNA isolation kit with strict adherence to the manufacturer's guidelines.

2.2 Polymerase chain reaction and microsatellite genotypin

Seven microsatellite markers (SAT3, SAT8, SAT12, SOL 3, SOL 8, SOL 28 and SOL 30) used in this study (Table 1). PCR carried out for the amplification of isolated DNA was prepared in a 25.00 µL cocktail mixture which contained 1.00 µL of DNA, 2.50 µL of 10×buffer, 1.00 µL of 25 mM dNTPs, 2.00 µL primer (1.00 µL of each forward and reverse), 0.20 µL of *Taq* polymerase, 2.20 µL of 25 mm/Mol Mg^{2+} and 16.10 µL distilled water. Denaturing temperature of 94°C (1 minute), and annealing temperature for the seven microsatellite primers ranged from 52°C to 60°C (Table 1). The initial extension was at 72°C (1 minute) followed by the final extension at 72°C (10 minutes). Products generated were subjected to 12% polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. Bands on gels were scored based on size of ladder with Gene Scan 3.1.2. Bands were designated as alleles and prepared into Excel Worksheet.

2.3 Ethics of animal experimentation

All procedures followed were done in strict adherence in accordance to the institutional animal welfare ethics.

2.4 Data analyses

Allele frequencies, observed heterozygosity (H_O), expected heterozygosity (H_E) mean number of alleles (MNA) and rare/unique alleles (R_A) were estimated for seven microsatellite markers using Microsatellite Analyser version 4.05 developed by Dieringer and Schlotterer [10]. F-statistics was obtained using the Genepop 4.1 program [11,12]. Gene flow was calculated using the formula suggested by Weir and Cockerham [13]. PIC for each marker and in each rabbit population was calculated using the formula long suggested by Botstein et al. [14]. Combined

Table 1. Sequences and the annealing temperature of seven microsatellite markers used in this study.

Locus	Primer Sequence	Annealing temperature (°C)
SAT3	F: 5'GGAGAGTGAATCAGTGGGTG3'	60
	R: 5'GAGGGAAAGAGAGAGACAGG3'	
SAT8	F: 5'CTTGAGTTTTAAATTCGGGC3'	55
	R: 5'GTTTGGATGCTATCTCAGTCC3'	
SAT12	F: 5'GGATTGGGCCCTTTGCTCACACTTG3'	58
	R: 5'ATCGCAGCCATATCTGAGAGAACTC3'	
SOL3	F: 5'ATTGCGGCCCTGGGGAATGAACC3'	58
	R: 5'TTGGGGGGATATCTTCAATTTCAGA3'	
SOL8	F: 5'CAGACCCGGCAGTTGCAGAG3'	60
	R: 5'GGGAGAGAGGGATGGAGGTATG3'	
SOL28	F: 5'TACCGAGCACCAGATATTAGTTAC3'	52
	R: 5'GTTGCCTGTGTTTTGGAGTTCTTA3'	
SOL30	F: 5'CCCGAGCCCCAGATATTGTTACCA3'	52
	R: 5'TGCAGCACTTCATAGTCTCAGGTC3'	

exclusion probability (CEP) across markers and populations was calculated using multiple products of each marker exclusion probabilities defined as:

CPE= 1 − (1 − P_{E1}) (1 − P_{E2})(1 − P_{E3}) …. … … … … … (1 − P_{EN}) [15],

Where PE1…PEN is exclusion probabilities of the seven microsatellite markers used.

3. Results and Discussion

The number of alleles observed across the seven microsatellite markers varied between 3 (SOL30) for New Zealand White and 15 (SOL28) for the Chinchilla rabbits. Mean number of alleles identified in the rabbit population was 11.142 ± 1.164, while for the four sub-populations, the values were 6.000 ± 1.024 for the New Zealand White, 8.857 ± 0.884 for Californian White, 8.143 ± 0.738 for New Zealand Red and 9.000 ± 1.069 for Chinchilla rabbit. The Chinchilla breed had the highest mean number of alleles of 9.000 ± 1.069 compared to other breeds (Table 2). The MNA observed over the seven loci for the four rabbit breeds are considered to be good indicators in defining the genetic variability within the population. The high overall MNA value recorded among the rabbit populations is indicative of great allelic diversity, which could have been influenced by crossbreeding or admixture among the rabbit populations (Table 2).

The seven microsatellite markers used for this study had at least one rare allele across the rabbit breeds. Rare alleles are alleles unique to a particular breed and/or population, thus, it is only fitting for such alleles to be used in the genetic identification of such population [16]. Emphasized the importance of high frequency of rare alleles in the genotyping and line identification of populations. Rare alleles observed in the different breeds for the markers used in this study were as follows: Californian White rabbit (6), New Zealand Red (4), New Zealand White (3) and Chinchilla (2).

The number of alleles observed ranged from 3-15 with the lowest number (3) produced by SOL30 being the least polymorphic marker and the highest number of alleles (15) produced by SOL28 being the most polymorphic loci. The range observed in this study is similar to 2-18 earlier reported in Tunisian rabbit populations [17]; (2-12) in the Egyptian and Spanish line rabbit populations [18], (4-12) in the pygmy rabbit breeds [19], (4-10) in the Egyptian rabbit populations [20]. The markers used for this study were appropriate since their polymorphisms were higher than the minimum of 4 alleles required

Table 2. Observed and expected heterozygosities, number of alleles, rare alleles, in each marker across rabbit populations.

Marker	Population	NA	RA	HO	HE
SAT3	NZW	4	0	0.8261	0.7402
	CAL	6	0	0.7619	0.8890
	NZR	6	1	0.8421	0.8852
	CHIN	7	0	0.8433	0.8901
SAT8	NZW	8	0	0.7368	0.7282
	CAL	8	0	0.8653	0.8150
	NZR	10	2	0.6111	0.7486
	CHIN	9	1	0.7539	0.8335
SAT 12	NZW	5	0	0.7224	0.8194
	CAL	8	1	0.8529	0.8731
	NZR	8	0	0.7436	0.7714
	CHIN	9	0	0.8454	0.8429
SOL3	CAL	8	1	0.7495	0.8635
	NZR	7	0	0.6523	0.8358
	CHIN	7	0	0.8888	0.8620
SOL8	CAL	11	2	0.8952	0.8340
	NZR	9	1	0.7912	0.8111
	CHIN	9	0	0.8636	0.7780
SOL28	CAL	13	1	0.8947	0.8546
	NZR	11	1	0.8235	0.8397
	CHIN	15	2	0.8947	0.8834
SOL30	CAL	8	1	0.7591	0.8036
	NZR	6	0	0.8334	0.8804
	CHIN	7	1	0.8662	0.8808

NZW 25: New Zealand White; CAL 25: Californian White; NZR 25: New Zealand Red; CHIN 25: Chinchilla; NA: Number of Alleles; RA: Rare Alleles; HO: Heterozygosity Observed; HE: Expected Heterozygosity.

for microsatellite markers to be used in the estimation of genetic diversity (Table 2).

Gene diversity, migrant rate, F-statistics, polymorphism information content, exclusion probabilities of marker and combined exclusion probabilities are summarized in Table 3. The PIC which takes into account the allele frequency per marker at a specific locus is a good indicator of genetic diversity evaluation.

The average PIC values were 0.7206, 0.8115, 0.7217 and 0.8214 for New Zealand White, Californian White, New Zealand Red and Chinchilla rabbit populations respectively. The PIC values were similar to those reported in earlier works on rabbit diversity studies using STRs, (0.625-0.796) in Asian rabbits [21] and (0.60-0.86) in Egyptian rabbit [20].

The values of the PIC recorded for this study showed that the microsatellite markers used were highly polymorphic and informative for genetic diversity studies, since all loci PIC value in this study was greater than the threshold value of 0.5 (i.e., the value of at which the microsatellite marker can be regarded as being informative) [14].

Average heterozygosity is an appropriate measure of genetic variability within a population because it takes into account all levels of genetic variation rather than just classify into 2 categories (monomorphic or polymorphic). Table 2 shows the observed heterozygosity which ranged from 0.7567 in New Zealand White to 0.8508 in Chinchilla rabbit breeds, while the expected heterozygosity or the gene diversity ranged from 0.7726 in New Zealand White to 0.8529 in Chinchilla. Heterozygosity range in this study was wider compared to that reported (0.53-0.62) by Zhu [22], (H_O, 0.61-0.63 and H_E, 0.63-0.65) [23]; (H_O, 0.39-0.58 and H_E, 0.30-0.56) [17]. High level of heterozygosity recorded in this study could be attributed to the mixed nature of the breeds. Furthermore, similarities in range were observed with reported studies of (H_O 0.37-0.79 and H_E 0.66-0.88), (0.63-0.72) [20,21]. The observed heterozygosity obtained in this study was lesser when compare to the expected heterozygosity in most population. This however could be attributed to one or more of the following: segregation of null alleles and improper scoring of heterozygotes [22-25].

Average genetic differentiation among breeds was 0.0479, which implies 96.60% of the total genetic variation was explained by individual variability (Table 3). Mean inbreeding coefficient of the individual relative to the sub-population (F_{IS}) was-0.0201, which indicate the existence of heterozygosity excess within the rabbit populations; however, this may be tested in further research. The low level of genetic differentiation (0.0479) was supported by the high level of gene flow (Nm), which suggests possible admixture among the rabbit populations (Tables 3 and 4). Exclusion probabilities of marker across populations (P_{EI}) ranged from 0.9000 (SOL28)

Table 3. Gene diversity, polymorphism information content, fixation indices, migrant rate, exclusion probabilities of marker and combined exclusion probabilities across markers and among four rabbit populations in Nigeria as revealed by seven microsatellite markers.

Marker	GD	PIC	FIS	FIT	FST	NM	PEI
SAT3	0.85	0.72	-0.0662	0.036	0.0958	2.369	0.94
SAT8	0.78	0.68	-0.0387	0.0407	0.0764	3.022	0.926
SAT12	0.82	0.8	0.0788	0.1044	0.0278	8.743	0.93
SOL3	0.83	0.78	-0.0681	-0.0395	0.0268	9.078	0.931
SOL8	0.79	0.77	-0.0041	0.0056	0.0097	25.523	0.924
SOL28	0.85	0.81	-0.0584	-0.0511	0.0069	35.981	0.907
SOL30	0.8	0.78	0.0154	0.1064	0.0924	2.4551	0.924
MEAN	0.82	0.76	-0.0201	0.0289	0.0479	12.453	
CPE							0.999999

GD: Gene Diversity; Nm: Gene flow; F_{IS}, FIT and F_{ST} are fixation indices; PIC: Polymorphism Information Content; PE1: Exclusion Probabilities of N number
of markers for one parent and both parents excluded; CPE: Combined Exclusion Probabilities for one and both parents excluded.

Table 4. Nei's genetic distance (below diagonal) and the proportion of shared alleles (above diagonal) of rabbit populations examined.

Population	New Zealand White	Californian White	New Zealand Red	Chinchilla
New Zealand White	00000	0.2863	0.3219	0.2722
Californian White	0.3258	00000	0.5139	0.5508
New Zealand Red	0.3108	0.1276	00000	0.5259
Chinchilla	0.3509	0.1005	0.1095	00000

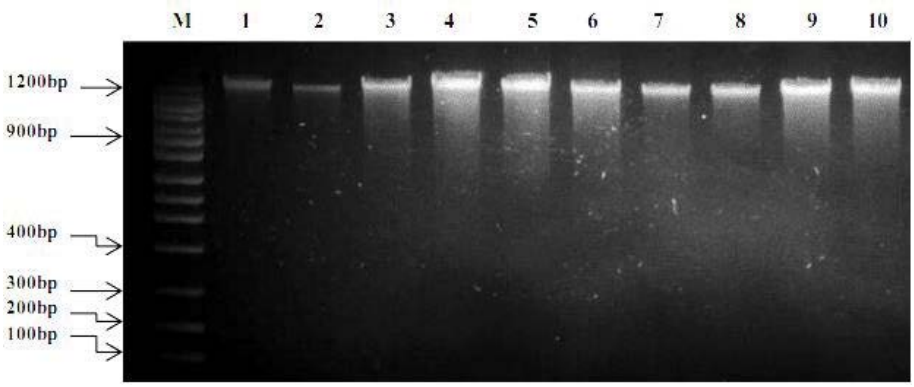

Figure 1. Electrophoreogram of purified DNA for quality verification.

to 0.9400 (SAT3), while the combined exclusion probabilities CEP across markers used was 9.99999 × 10^{-1} when one parent was excluded (Table 3 and Figure 1).

4. Conclusion and Applications

The rabbit is believed to have been discovered by Phoenicians when they reached the shores of Spain about 1000BC. The historical record of rabbit keeping in Nigeria is not available; despite speculations of the commencement of rabbit rearing with the advent of slave-trading and European invasion into Africa, when most exotic agriculturally important crops and livestock were introduced. There are many breeds of rabbits introduced to Nigeria from Europe and other West African countries, since rabbits are not native to Nigeria compared to the African hare (*Lepus sp.*).

However the four breeds used in this research are the commonest of them all, an attribute which could be said is due to their adaptation to the Nigerian environment (climatic, disease resistance, management and so on) Microsatellite markers used in this study were found to be highly polymorphic and informative based on PIC>0.5. The genetic characterization as revealed by the microsatellite markers showed that the four rabbit breeds has more within breed variation than between breed variation. Similarly, the low values of genetic differentiation with regard to the inbreeding estimates indicate relatively high outbreeding among the four rabbit breeds.

High values of mean number of alleles across loci and the expected heterozygosity recorded across loci in the rabbit populations indicates high genetic diversity among the rabbit populations in Nigeria. The combined power of the seven microsatellite markers used was 0.999999, thus signaling the informativeness and effectiveness of the markers in parentage verification of common rabbit breeds in Nigeria.

The genetic distance is a population genetic parameter used to ascertain the degree of relatedness between sub-groups in a population. The values for the genetic distance measures ranges from 0-1, in which values tending towards 0 indicate closely related individuals, while value that tend towards 1 indicate distant related individuals. The Nei's genetic distance was used to ascertain the degree of relationship among the four rabbit populations in Nigeria. The Californian White and the Chinchilla rabbit populations revealed the closest relationship, while the farthest relationship was recorded between the New Zealand White and Chinchilla breeds of rabbit. The closely relatedness between the Chinchilla and the Californian White in Nigeria, is indicative of a higher level of intermixing between both breeds, while, the distant related New Zealand White, indicated possible isolation from other breeds.

Furthermore, the availability of microsatellite markers for genetic diversity studies for the rabbit continues to be limited as compared to other livestock species, whose microsatellite markers have been elaborately documented on recognized scientific platforms (ISAG, FAO), thus, more genetic research needs to be done to encourage the provision of recommended microsatellite markers for advance experimentation on the rabbit population characterization. Improvement of the rabbit stock can only be realized when up to date genetic and genomic data are available, and such data are accessible only through genetic or genomic characterization procedures, hence the genetic diversity estimates from this study which is the first of its kind in rabbit populations in Nigeria. Conducted with the sole aim of aiding the facilitation of rabbit conservation, provision of more information which would encourage the sensitization of subsistent rabbit farmer's on the need for rabbit breeds preservation, pure breeding and crossbreeding methodologies to curb indiscriminate mating, in order to ultimately achieve a well-defined breed improvement goal.

5. Contribution of Authors

Adewunmi O Omotoso and Olajide Olowofeso

designed the work. Adewunmi O Omotoso carried out the field work. Adewunmi O Omotoso did the laboratory work. Matthew Wheto advised on the laboratory work. Adewunmi O Omotoso and Olajide Olowofeso did the statistical analysis. Adewunmi O Omotoso and Olajide Olowofeso drafted the manuscript. Olajide M Sogunle proof read the drafted manuscript. All authors read and approved the final manuscript.

6. Conflict of Interest Statement

I wish to clearly state that this article titled "Genetic Variation among Four Rabbit Populations in Nigeria using Microsatellite Marker" has received substantive contributions from the following authors (A. O. Omotoso, O. Olowofeso, M. Wheto, and O. M. Sogunle) with no conflict of interest.

References

[1] Vietmeyer ND. (1985). Potential for micro livestock in the developing countries. *JApp Rabbit Res*. **8**:10-11.

[2] Irlbeck NA (2001).How to feed the rabbit (*Oryctolagus cuniculus*) gastrointestinal tract. *J Animal Sci*. **79**:343-346.

[3] Talle SB, Chenyabuga WS, Fimland E, et al. (2005). Use of DNA Technologies for the Conservation of Animal Genetic Resources. *J Scand Animal Agri*. **55**:1-8.

[4] Galal OA, Rehan M, Abd El-Karim RE. (2013).Analysis of genetic diversity within and among four rabbit genotypes using biochemical and molecular genetic markers. *African J Biotech*. **12**:2830-2839.

[5] Lukefahr SD. (2000). The National Rabbit Project population of Ghana: a genetic case study. In: *Workshop on Developing Breeding Strategies for Lower Input Animal Production Environments,* September 22-25, 2000. Food and Agriculture rganization (FAO) of the United Nations, Rome. ICAR Tech. Series No. 3:307-318.

[6] Groenen MA, Cheng HH, Bumstead N, et al. (2000). A consensus linkage map of the chicken genome. *Genome Resource*.**10**:137-147.

[7] Olowofeso O, Wang JY, Shen JC, et al. (2005). Estimation of the cumulative power of discrimination in Haimen chicken populations with ten microsatellite markers. *Asian-Australasian J Animal Sci*.**18**:1066-1070.

[8] Tadano R, Nishibori M, Nagasaka N, et al. (2007). Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analyses. *J Poultry Sci.* **86**:2301-2308

[9] Onifade AA, Abu OA, Obayan RI. (1997). Rabbit production in Nigeria; some aspects of current status and promotional strategies. *J World Rabbit Sci*.**7**:51-58.

[10] Dieringer D, Schlotterer C. (2003). Two distinct modes of microsatellite mutation processes; evidence from the complete genomic sequences of nine species. *Genome Res*. **13**: 2242-2251.

[11] Raymond M, Rousset F. (1995).population genetics software for exact tests and ecumenicism. *J Heredity*. **86**:248-249.

[12] Rousset F. (2008). Genepop'007: A complete reimplementation of the Genepop software for Windows and Linux. *Mol Ecology Resour*. **8**:103-106.

[13] Weir BS, Cockerham CC. (1984). Estimating F-statistics for the Analysis of Population Structure. *J Evol*. **38**: 1358-1370.

[14] Botstein D, White RL, Skolnick M, et al. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *America Human Genet*. **32**:314-331.

[15] Řehout V, Hradecka E, Čitek J. (2006).Evaluation of parentage testing in the Czech population of Holstein cattle. *Czechosl Animal Sci.* **51**: 503-509.

[16] Toro M.A, Fernández J, Caballero A. (2008). Molecular characterization of breeds and its use in conservation. *Conservation Biol*. **14**:1843-1851

[17] Ben-Larbi M, San-Cristobal M, Chantry-Darmon C, et al. (2014). Population structure in Tunisian indigenous rabbit ascertained using molecular information. J *World Rabbit Sci*. **22**:223-230.

[18] Grimal A, Safaa HM, Saenz-de-Juano MD, et al. (2012). Phylogenetic Relationship among Four Egyptian and One Spanish Rabbit Populations Based On Microsatellite Markers. World Rabbit Science. 177-181.

[19] Estes-zumpf WA, Rachlow JL., Waits LP. (2008). Ten Polymorphic Microsatellite Markers for the Pygmy Rabbit (*Brachylagus idahoensis*). *J Mol Ecology Resour*. **8**:360–362.

[20] El-Aksher SH, Sherif HS, Khalil MH. (2016). Comparative Genetic Analysis among Moshtohor Line Rabbits and their Parental Lines using Microsatellite Markers. 3rd International Conference on Biotechnology Applications in Agriculture (ICBAA), Benha University, 5-9 April 2016 Egypt.

[21] Xin-Sheng W, Tian-Wen W, Hui-ling Z. (2008). Correlation Analysis of Wool Yield in Wan line Angora Rabbits using Microsatellite DNA Markers. *J Biol Sci*. **8**: 679-682.

[22] Zhu YF, Zhang JB, Ren WZ, et al. (2004). Genetic Variation Within and Among Five Rabbit Populations Using Microsatellite Markers. Proceedings of the 8th World Rabbit Congress–September 7-10, 2004 – Puebla, Mexico.pp 181-185.

[23] Queney G, Ferrand N, Marchandeau S, et al. (2000). Absence of a genetic bottleneck in a wild rabbit (*Oryctolagus cuniculus)* population exposed to a severe viral epizootic. *Molecul Ecol*. **9**:1253-1264.

[24] *http://www.fao.org/docrep/t1690e/t1690e00.html*

[25] Onifade AA, Abu OA, Obayan RI, et al.(1999). Rabbit Production in Nigeria; Some Aspects of Current Status and Promotional Strategies. *J World Rabbit Sci.* **7**:51-58