PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

Session
Breeding and Genetics

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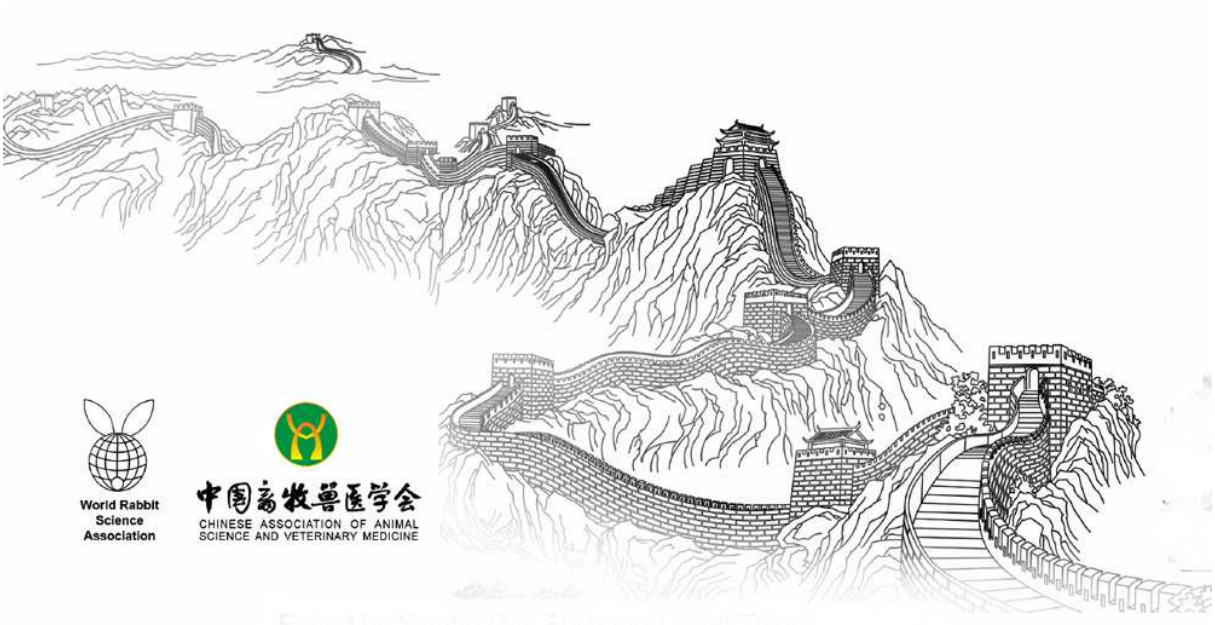
Full text of the communication

How to cite this paper :
Orheruata A.M., Imasuen A.J., Ichekor C., 2016 - Assessing the genetic similarities and distance among rabbit populations using the random amplified polymorphic DNA (RAPD) technique *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 99-102.*



ASSESSING THE GENETIC SIMILARITIES AND DISTANCE AMONG RABBIT POPULATIONS USING THE RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNIQUE

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ABSTRACT

The study was carried out using the random amplified polymorphic DNA (RAPD) technique to assess the genetic similarities and distance among rabbit populations. 100 rabbits from four strains and a composite population were used for the study. The animals include New Zealand White, Californian, Rex, Dutch and the Composite population. Results obtained showed genome of Californian strain could not be amplified by OPA-10. A within- and between-strain band-sharing frequency of less than one but above zero was obtained and was significant ($P < 0.05$). The BSF values within strain were REX (0.98 ± 0.01), NZW (0.88 ± 0.02), DUT (0.88 ± 0.02) and COM (0.81 ± 0.04). Between strains BSF values were 0.88 ± 0.02 between NZW and REX strains, 0.85 ± 0.02 between COM and DUT strain, 0.84 ± 0.01 between RX and DUT strains, 0.77 ± 0.02 between NZW and DUT strains, 0.75 ± 0.01 between REX and COM, and 0.72 ± 0.02 between NZW and COM. The Nei's genetic distance (D) was highest between NZW and COM (D = 0.3285) and least between NZW and REX (D = 0.1278). The results showed a low level of genetic variation. The study suggests that RAPD can be successfully utilized to detect genetic variation among rabbit strains.

Key Words: Genetic similarities, Distance, Rabbits, RAPD-DNA

INTRODUCTION

In Nigeria, rabbit production is on a small scale and is predominantly in composite population described by Lukefahr (1998) as non-standard. In recent times, there are farms with standard breeds though they are smaller in size but have the phenotypic feature of standard breeds. The question is are these breeds genetically different from the non-standard population? Such question can only be answered by look at them at the gene level. A lot of molecular markers have been used for several purposes like genetic analysis of inbred strains of rabbit (*Oryctolagus cuniculus*) (Van Haerigen, *et al.*, 2001). Williams *et al. (*1990) *used t*he random amplified polymorphism DNA (RAPD) technique to determine genetic relationship among organisms. However, in Nigeria there are no reports available for detection of genetic variability in rabbit breeds using RAPD markers. Hence, the RAPD-PCR was used as a tool to assess the similarity and the genetic distances among rabbit breeds in Nigeria.

MATERIALS AND METHODS

Experimental Animals and Management

A total of 100 rabbits (mixed sexes) comprising of rabbit populations with phenotype of New-Zealand White, Rex, Dutch and Californian sourced from a reputable livestock farm and composite rabbits population in the University of Benin Teaching and Research Farm were used for the study.

Blood Collection and DNA Extraction

3ml blood was collected from 5 individuals in each rabbit population into a 5ml sterilized vacutainer tube containing EDTA as anticoagulant. Genomic DNAs were isolated from the blood samples and purified using QIAamp Mini Spin Kit (250) procedure. The quantity of DNA was checked through UV spectrophotometer and an absorbance of OD2.8/OD1.5 =1.867 approximately 1.9 was got as the ratio of 260/280. DNA concentration of 1to 2 ng/µl was accepted to be pure without contamination. Each DNA sample was transferred to the PCR machine for amplification.The DNAs were amplified using a Thermal

Cycler and the products were subjected to Gel electrophoreses to detect bands used to determine band sharing frequency (BSF) and genetic distance (D). Data were subjected to statistical analysis to obtain descriptive and inferential statistics.

Random Amplified Polymorphic DNA (RAPD)-PCR

About 40 decamer RAPD primers with 60 – 70% guanine + cytosine (GC) content (e.g OPA – 1 to OPA – 20 and OPB-20) were screened on pooled rabbit DNA and the primer with distinct polymorphism and more number of bands was selected. The selected primer (OPA-10) with sequence GTGATCGCAG and 60% of guanine + cytosine content was used. 20µl of the PCR product was loaded in 1.5% agarose and run at 100v. The gel photograph was captured through gel documentation system.

Analysis of RAPD Data

Only distinct and prominent bands were scored for the estimation of genetic parameters. The presence and absence of RAPD band was recorded as "1" and "0", respectively and thereafter analyzed for band sharing frequency and genetic distances within and between rabbit populations.

Band Sharing Frequency (BSF)

Band sharing frequency (BSF) was used to calculate the genomic expression of the rabbits using the procedure of Jeffery and Morton (1987) expressed as
BSF = 2Nab/(Na + Nb), Where Nab is the number of bands common to a & b individual, Na is the number of bands present in the animal a, while Nb is the number of bands present in the animal b. The BSF values within and between populations was subjected to analysis of variance (ANOVA) using General Linear Procedure of SAS (2014). Significant means were separated using Duncan Multiple Range Tests.

Genetic Distance (D)

The genetic distance (D) was calculated using Nei (1972) standard genetic distance equation expressed as: D = -In (F). Where F is an estimate of similarity, which is based on the fraction of shared RAPD markers between populations; F was calculated using the formula:
$F = 2X_{1,2} / X_1 + X_2$. Where $X_{1,2}$ is the number of amplified DNA fragments with the same molecular weight found in both populations, X_1 is the total number of fragments found in one population, and X_2 is the total number found in the other. This is the same as the BSF formula given above.

RESULTS AND DISCUSSION

Rapd Profile In Rabbit Populations

The RAPD profile (fingerprints) of the rabbit included DNA ladder to facilitate scoring of the RAPD fingerprint. The RAPD profile showed both distinct (bold) bands and invisible (faint) bands in the gel photograph. RAPD allele frequency was obtained by direct counting. The amplified DNA fragments showed bands that varied from 8 to 10 with a size range varying from 100bp to 1500bp in all the strains except Californian strain.

Band Sharing Frequency (Bsf)

The band sharing frequency values within strain were slightly higher for REX (0.98 ± 0.01) and least in COM (0.81 ± 0.04). The value for NZW was not-significantly different ($P > 0.05$) from that of DUT (Table 1). The between strains value of 0.72 ± 0.02 was obtained for NZW-COM and the highest value of 0.88 ± 0.02 for NZW-REX strains. There was significant difference ($P < 0.05$) in the BSF of the strains. There was low level of genetic diversity among the individuals.

Table 1: Band Sharing Frequency (BSF) within and between Populations (Mean±SEM) using OPA-10

		Within			Between
Population	**No**	**BSF (Mean ± SEM)**	**Populations**	**No**	**BSF (Mean ± SEM)**
NZW	10	0.88 ± 0.02^{b}	NZW-REX	5	0.88 ± 0.02^{a}
REX	10	0.98 ± 0.01^{a}	NZW-COM	5	0.72 ± 0.02^{c}
COM	10	0.81 ± 0.04^{c}	NZW-DUT	5	0.77 ± 0.02^{b}
DUT	10	0.88 ± 0.02^{b}	REX-COM	5	0.75 ± 0.01^{bc}
			REX-DUT	5	0.84 ± 0.01^{a}
			COM-DUT	5	0.85 ± 0.02^{a}

NZW: New Zealand White, REX: Rex, COM: Composite population, DUT: Dutch. Means bearing same superscripts did not differ significantly; $p < 0.05$: significant.(DMRT)

Genetic Distances Between Rabbit Strains Using Opa – 10

The genetic distance (D) value was between 0.13 and 0.33 (Table2). NZW and COM had the highest distance and the least between NZW and REX strains.

Table 2: Genetic distances between rabbit strains using OPA-10

Strains	NZW	REX	COM	DUT
REX	0.13			
COM	0.33	0.29		
DUT	0.26	0.17	0.16	

NZW: New Zealand White, RX: Rex, COM: Composite population, DUT: Dutch,

The results of both intra and inter populations band sharing frequency indicated that the majority of the fingerprints were shared, to different extents, by individuals in the different populations. Since each RAPD fingerprint may represent or be linked to a separate allele, any shared fingerprint may be contemplated as a product of the same allele. Hence, the level of similarity based on allele sharing was high both within and between populations. However, RAPD fingerprints that are not shared are, therefore, powerful tools for discriminating different populations or strains phenotypically. The results of this study point to the fact that such discriminatory fingerprints were found more in the Composite population and scanty in the other strains. The high level of variability observed within the Composite population compared to the other populations suggested that a great deal of crossing may have occurred among the various rabbit strains in Nigeria leading to individuals with mixed germplasm.

According to Nei (1975), heterozygosity is a good measure of genetic diversity of polymorphic loci. The high band sharing frequency values recorded within-strain and between-strains suggested low heterozygosity existing among these strains. Thus, higher genetic diversity was displayed within the Composite population than within the other three populations and the high variation might be as a result of the random mating among the animals of the Composite population. This supports the notion that populations with high level of band sharing values have lower level of genetic differences hence the genetic diversity in the Composite population is wider than in the other strains. The results also indicated that the low variation within-strain as compared to inter-strain was found to be in agreement with the findings of Mamuris *et al*. (2002).

In this study, the genetic distance observed was higher compared to that of Rangoju *et al*. (2007) but was however lower than that of El-Bayomi Kh *et al*. (2013). The major reason that could have led to these genetic variations might be due to different strains (or species), breeding method and selection (natural or artificial) taking place in these different geographical and climatic conditions which could have caused variation in the gene pool. The diversity among the Nigeria rabbit strains as observed from this study was low probably as a result of strong inbreeding that may have occurred. Therefore, as expected, these strains showed less genetic diversity. The selection and random mating could have altered the allele frequency by reducing the likelihood that one or more genotypes would contribute to the next generation (Delany, 2003). However, the findings of this study were consistent with the reports of Mamuris *et al*., 2002; Rangoju *et al*., 2007; and El-Bayomi Kh *et al*., 2013.

CONCLUSION

The study has demonstrated that by using random primers of arbitrary nucleotide sequences, it was possible to show fingerprints of individual animals and populations. The use of RAPD marker technology to unveil the differences that existed in the DNA sequence of these rabbits in this study is therefore not out of place.

ACKNOWLEDGEMENT

The study was sponsored by the grant from the University of Benin Research and Publication Directorate.

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