World Rabbit Sci. 2007, 15: 3 - 8

ASSESSMENT OF GENETIC VARIABILITY AMONG RABBIT BREEDS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)-PCR

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Abstract: Random amplified polymorphic DNA (RAPD) technique was employed to assess the genetic variation and phylogenetic relationship among three broiler rabbit breeds. Ten individuals from each rabbit breed viz. White Giant (WG), Soviet Chinchilla (SC) and Grey Giant (GG) was taken for the study. Initially, 40 RAPD primers were screened, of which six primers were found polymorphic and they were further utilized to assess the genetic variability among these breeds. The band sharing frequencies (BSF) were computed within and between breeds. The overall BSF value within breed was highest in WG (0.846±0.02) and GG (0.846±0.01), while lowest in SC (0.818±0.02). However, between breeds, BSF value was found higher in SC-GG (0.805± 0.01) followed by WG-SC (0.792±0.02) and WG-GG (0.790±0.02). Overall, there was no significant difference (P>0.05) in BSF values within and between breeds. The BSF value indicated low genetic variability within the breed as compared to between breeds. The Nei's genetic distance (D) was found highest between WG-GG (D=0.1605) followed by WG-SC (D=0.1403) and SC-GG (D=0.1295). The phylogenetic relationship among breeds was analyzed and dendrogram revealed that SC and GG are more closer, while WG-GG are distant to each other. The study suggests that RAPD can be successfully utilized for detecting genetic variation among rabbit breeds.

Key words: Rabbit, breed, RAPD, genetic variability.

INTRODUCTION

Rabbit has its importance as supplier of meat, and it is widely accepted thought out the world for human consumption (Colin and Lebas, 1996). Rabbit rearing has been practiced on commercial scale in countries like Italy, France, Ukraine, China, Spain and Russia since earlier times. However, in India broiler rabbits have become as source of food only during the last two-three decades. The adaptation of rabbit populations to climatic conditions may represents a genetic variability in their gene pool and this variability causes genetic differentiations among rabbit breeds/populations. The genetic diversity of reared and wild animals is declining and this warrants serious attention of the researchers and conservators to find out ways to conserve the diversity at the maximum level. The estimation of genetic variability of a species is an important criterion for its conservation and further genetic improvement. The genetic variability can be assessed by nuclear markers such as allozymes. These are conservative genetic markers, evolving slowly but do not have much resolving power to reveal population differentiation. The genetic variability in brown hare populations (*Lepus europaeus*) was analyzed using allozymes by previous works (Hartl *et al.,* 1992, 1993; Suchentrunk *et al.,* 2000), but they could not reveal much genetic differentiation among hare population. Subsequently, genetic differentiation among hare populations was assessed by mitochondrial (mt) DNA by several workers (Parez-Suarez *et al.,* 1994; Pierpaoli *et al.,* 1999), revealing a highly haplotype diversity within and among wild brown

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Received *March 2006* - Accepted *November 2006*.

hare populations (Mamuris *et al.,* 2001). Other molecular markers like AFLP (amplified fragment length polymorphism) have been used for several purposes like genetic analysis of inbred strains of rabbit (*Oryctolagus cuniculas*), for quantitative traits (Van Haeringen *et al.*, 2001) and microsatellites for diversity analysis in wild rabbits (Zenger *et al.*, 2003).

On the other hand, random amplified polymorphic DNA (RAPD) technique is highly informative for understanding genetic relationship among the organisms, but it has low reproducibility (Williams *et al.,* 1990). RAPD markers are the randomly amplified target regions of less functional part of the genome that do not strongly respond to selection on the phenotype level. Such amplified regions may accumulate more mutations thereby offering a wider potential in assessing the interbred/population genetic differentiation. RAPD-PCR has been used as a tool to assess the genetic variability and phylogenetic relationship in hare populations (*Lepus europaeus*) in Greece and European countries (Mamuris *et al.,* 2002). However in India, there is no report available for detection of genetic variability in rabbit breeds using RAPD markers. Hence, the RAPD-PCR has been used as a tool to assess the genetic variability and phylogenetic relationship among broiler rabbit breeds adapted in the semi-arid climate of the Rajasthan State of India.

MATERIAL AND METHODS

Animals

A total 30 unrelated individuals (irrespective of sex) of broiler rabbits viz. White Giant (WG, n=10), Soviet Chinchilla (SC, n=10) and Grey Giant (GG, n=10) were taken for the study. These breeds were received in 1978-79 from former USSR under the Indo-USSR protocol for agricultural development and initially maintained in the temperate climate of Kullu Valley at Garsa in Himachal Pradesh, India. Subsequently in 1983, these rabbits were transferred to the Central Sheep and Wool Research Institute (CSWRI), Avikanagar, located in the semi-arid region of Rajasthan. The CSWRI, Avikanagar is located at 75^0-28'E longitude and 26^0-26'N latitude and an altitude of 320 m above mean sea level. The location is typically hot semi-arid with yearly minimum and maximum temperatures ranges between 4^0C and 46^0C, respectively. These animals were maintained under standard farm managemental practices developed at CSWRI for rabbit rearing. The animals were fed lucerne hay *ad libitum* in the morning and concentrate feed pellets in the evening (100g/head/day), with watering 2-4 times/day depending upon the season.

Blood collection and DNA extraction

Approximately 2 ml blood was collected from each individual (from the central artery vein of the ear) in 15 ml centrifuge tubes containing 350 µl of ACD (Citric acid, Sodium citrate, Dextrose) as anticoagulant. Genomic DNA was isolated using Phenol-Chloroform extraction method with slight modifications (Clamp *et al.,* 1993). DNA pellet was air-dried and dissolved in 50 µl of 0.1 × TE (pH 8.0) and stored at 4°C till further use. The quantity of DNA was checked through UV spectrophotometer, while quality of DNA was ascertained through agarose gel electrophoresis. The intact DNA showing no smearing was selected for further analysis.

Random amplified polymorphic DNA (RAPD)-PCR

Initially, 40 decamer RAPD primers with 60-70% guanine + cytosine (GC) content (OPA-1 to OPA-20 and OPB-1 to OPB-20) were screened on pooled rabbit DNA. All primers were purchased from Operon Technologies Inc, Germany. Finally 6 primers were selected, based on their distinct polymorphism and more number of bands, and used for all individuals. The list of primers and their sequence has been shown in Table 1. The PCR reaction mixture comprised as follows: 1 × PCR buffer (containing $(NH_4)_2SO_4$), 1.5 mM $MgCl_2$, 100 µM dNTPs mix, 1U Taq DNA Polymerase (MBI Fermentas), 25 ng of each primer and 25 ng of template DNA in 20 µl reaction volume. The amplification was performed in

Table 1: List of random amplified polymorphic DNA (RAPD) primers and their nucleotide sequence.

No.	Nomenclature	Sequence (5' → 3')	G+C (%) content
1	OPA-1	CAGGCCCTTC	70%
2	OPA-8	GTGACGTAGG	60%
3	OPA-10	GTGATCGCAG	60%
4	OPA-18	AGGTGACCGT	60%
5	OPB-3	CATCCCCCTG	70%
6	OPB-5	TGCGCCCTTC	70%

Thermal Cycler (Master Cycler Gradient, Eppendorf, Germany) with the following amplification conditions: initial denaturation at 94^{0}C for 2 min, followed by denaturation at 94^{0}C for 1 min, annealing at 40^{0}C for 1 min, extension at 72^{0}C for 2 min for 40 cycles and final extension at 72^{0}C for 5 min. The 20 µl PCR product was loaded in 1.5% agarose gel and run at 100V. Gel photograph were captured through gel documentation system (Figure 1).

Analysis of RAPD data

Only distinct and prominent bands were scored for estimation of genetic parameters. The presence and absence of RAPD band was recorded as "1" and "0", respectively. The binary coded characters (1,0) were used for the genetic analysis. The following statistical analyses were carried out for assessing the genetic variability and relatedness within and between breeds.

Band Sharing Frequency (BSF)

Band Sharing Frequency (BSF) was calculated as an expression of animals from either the same or different breeds (Jeffery and Morton, 1987) using the following formula.

$$BSF = 2Nab / (Na + Nb)$$

whereas, Nab is the number of bands common to a and 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 breeds were analyzed by analysis of variance (ANOVA) using

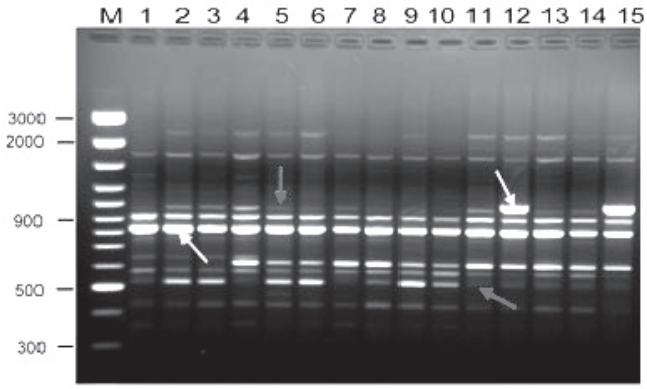

Figure 1. RAPD profile in rabbit breeds using OPA-10 primer. Lane 1-5: White Giant, lane 6-10: Soviet Chinchilla, lane 11-15: Grey Giant, M; 100bp ladder. The white color arrows show the distinct bands, while grey colour arrows showing invisible bands (faint) in the gel photograph.

SPSS 14 program. Significant differences between means were detected using Duncan's multiple range tests (Kramer, 1957).

Genetic Distance (D)

Genetic distances are designed to express the genetic differences between two populations as a single number. If there are no differences, the distances could be set to zero, whereas if the population have no allele in common at any locus the distance may be set equal to its maximum value, 1. The genetic distances (D) were calculated by POPGENE software (Yeh *et al.,* 1999) using Nei (1972) standard genetic distance equation.

Phylogenetic relationship

The phylogenetic relationship among rabbit breeds was analyzed by generating the phylogenetic tree by Nei (1972) genetic distances using UPGMA analysis through POPGENE software (Yeh *et al.,* 1999).

RESULTS AND DISCUSSION

In the present study, RAPD technique was used to assess the genetic variability and phylogenetic relationship among three rabbit breeds adapted in the semi-arid climate of Rajasthan. Six primers were used in amplification and produced the bands in the range of 200 to 2000 bp in all the breeds. The maximum numbers of bands were found in GG (13.8 ± 1.0) followed by SC (13.3 ± 0.6) and WG (12.5 ± 0.6) using OPB-5 primer. Primer OPB-5 gave the maximum number of bands (13.2 ± 0.4), while minimum numbers of bands were obtained using OPB-3 primer (6.4 ± 0.2) in all the breeds. Similar type of the study was conducted by Mamuris *et al*. (2002), they used RAPD primers (OPA-02, OPA-9, OPA-10, OPA-20 and OPF-1) for assessment of genetic variability among brown hare *(L. europaeus)* population from different geographical regions. In his study, all primers produced polymorphic bands in the range of 5 to 11, which is very similar to our study. In the present study, individual primer failed to produce specific markers for any breeds irrespective of sex and OPA-9 could not be amplified in any breed during the study. The data is presented in Table 2.

In Table 3, overall BSF values within breed were found slightly higher for WG (0.846 ± 0.01) and GG (0.846±0.01) than for SC (0.818 ± 0.02), but the difference was non significant ($P>0.05$). Although, significant differences ($P<0.05$) in BSF values within breed were detected using OPA-1 and OPB-5 primers. The BSF value was also analyzed between breeds, being the highest (0.805 ± 0.02) between SC-GG, followed by WG-SC (0.792 ± 0.02) and WG-GG (0.790 ± 0.02) (Table 4). Overall, there was no significant difference in the BSF values between breeds ($P>0.05$), however the significant difference ($P<0.05$) was reported using OPA-10 primer only. The OPB-3 primer revealed the highest value, while OPA-8 is the lowest in all breed combinations (WG-SC, SC-GG and WG-GG). It is assumed that the

Table 2: Average number of bands per primer in different rabbit breeds (mean ± SEM)

Breeds	N	OPA-1	OPA-8	OPA-10	OPA-18	OPB-3	OPB-5
WG	10	10.7±0.5 (9-13)	9.8±1.1 (7-16)	10.1±0.5 (8-13)	9.3±1.0 (5-13)	6.3±0.3 (5-8)	12.5±0.6 (10-16)
SC	10	12.1±0.4 (10-14)	9.2±0.9 (7-17)	8.9±0.5 (7-12)	9.4±1.0 (4-14)	6.4±0.4 (5-8)	13.3±0.6 (9-16)
GG	10	12.5±0.2 (12-13)	8.7±0.7 (6-13)	9.3±0.5 (7-12)	10.2±0.8 (6-14)	6.5±0.4 (5-8)	13.8±1.0 (9-17)
Overall	30	11.7±0.3	9.2±0.5	9.4±0.3	9.6±0.5	6.4±0.2	13.2±0.4

WG: White Giant, SC: Soviet Chinchilla, GG: Grey Giant, N: number of individuals, values in brackets are the range of bands in the breed.

Table 3: Band sharing frequency (BSF) within breed (mean ± SEM)

Primers	No. of observation	WG	SC	GG
OPA-1	20	0.884±0.01[a]	0.929±0.01[b]	0.910±0.01[ab]
OPA-8	20	0.745±0.03	0.646±0.04	0.736±0.03
OPA-10	20	0.812±0.02	0.875±0.01	0.836±0.03
OPA-18	20	0.810±0.03	0.724±0.04	0.792±0.03
OPB-3	20	0.937±0.01	0.962±0.01	0.933±0.01
OPB-5	20	0.883±0.02[a]	0.820±0.01[b]	0.877±0.02[ab]
Average	120	0.846±0.02	0.818±0.02	0.846±0.01

WG: White Giant, SC: Soviet Chinchilla, GG: Grey Giant. Same superscripts did not differ significantly. $P<0.05$: significant.

sequence of OPB-3 primer is frequently observed in all the breeds and shares maximum number of bands, while primer OPA-8 was found less polymorphic within and between breeds. The results indicated that primers revealed low heterozygosity within breed as compared to interbreed. The similar trend was also reported by (Mamuris *et al.*, 2002) when genetic identity was found higher within population as compared to between populations of brown hare from different regions.

The overall genetic distance was highest between WG-GG (D = 0.1605) followed by WG-SC (D = 0.1403) and SC-GG (D = 0.1295) using six primers. However, individual primers gave more variable results between breeds. The study suggests that SC-GG breeds are genetically more closer, while WG-GG individuals are most distant. In our study, the genetic distance observed was higher as compared to the results shown by Mamuris *et al*. (2002), which indicated less genetic distances (average of D = 0.009) in wild Greek hare populations. These variations might be due to the different genus/species and different geographical climatic conditions, which cause variability in the gene pool.

The tree constructed by POPGENE software revealed that SC individuals clustered with GG, whereas WG neither clustered with SC nor GG individuals and falls into a separate clade (Figure 2). The results generated from both methods showed that SC is more closer to GG individuals and they belong to the same clade. Mamuris *et al*. (2002) found that reared hare population was similar to the individuals from the European countries (Austria, Poland, Germany, and France) as compared to the six wild populations of Greece. Within Greece the distribution of the wild population did not follow any geographical trend because their genetic divergence seems to be independent to the geographical

Table 4: Band sharing frequency between breeds (mean ± SEM)

Primers	No. of observation	WG-SC	SC-GG	WG-GG
OPA-1	10	0.857±0.02	0.812±0.04	0.859±0.02
OPA-8	10	0.695±0.06	0.675±0.05	0.655±0.06
OPA-10	10	0.749±0.03[a]	0.840±0.03[b]	0.779±0.02[ab]
OPA-18	10	0.751±0.07	0.767±0.04	0.746±0.04
OPB-3	10	0.916±0.01	0.944±0.02	0.931±0.02
OPB-5	10	0.785±0.02	0.799±0.03	0.782±0.03
Average	60	0.792±0.02	0.805±0.02	0.790±0.02

WG: White Giant, SC: Soviet Chinchilla, GG: Grey Giant. Same superscripts did not differ significantly. $P<0.05$: significant.

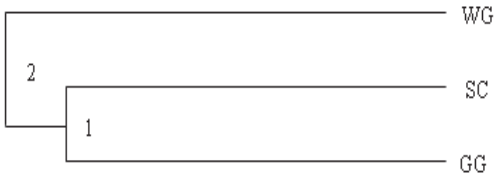

Figure 2. Phylogenetic tree's based on Nei (1972) genetic distances using UPGMA analysis through neighbor procedure of Phylip Version 3.5 (POPGENE software).

distance. Analysis of the genetic diversity among rabbit breeds through RAPD-PCR is very limited in India or elsewhere, therefore, there is no report available to compare our findings with other study except few. The present study suggests that RAPD still can be used as a tool to understand the genetic variability and phylogenetic relationship among rabbit breeds, but more number of samples and various sets of primers are required to further find out the genetic relationship in rabbit breeds.

Acknowledgement: The authors are highly thankful to the Director of CSWRI Avikanagar for providing the facilities required for carrying out the study. Mr. Sitaram, lab attendant is also acknowledged for his help and support.

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