Journal of Animal Research: v.6 n.4, p. 597-602. August 2016

DOI: 10.5958/2277-940X.2016.00069.3

Molecular Characterization of New Zealand White and APAU Black Rabbits using Microsatellite Markers

N. Govardhana Sagar[1]*, S. Sai Reddy[1], B. Ramesh Gupta[1] and M. Mahendar[2]

[1]Department of Animal Genetics and Breeding, College of Veterinary Science, Rajendranagar, SPVNRTSUVAFS, Hyderabad, INDIA

[2]Department of Livestock Production and Management, College of Veterinary Science, Rajendranagar, SPVNRTSUVAFS, Hyderabad, INDIA

**Corresponding author: NG Sagar; Email: sagar8930@gmail.com*

Received: 14 March, 2016 **Accepted:** 07 June, 2016

ABSTRACT

Genetic diversity among two genetic groups (New Zealand White and APAU Black) was investigated with a set of 20 microsatellite markers in the present study. The results showed that out of 20 microsatellites, 12 rabbit specific markers were successfully amplified by PCR which were highly polymorphic. A total of 257 alleles were observed across the 12 loci amplified. Number of alleles ranged from 5 to 14 and 5 to 16 in New Zealand White and APAU Black populations, respectively. The overall mean values of observed heterozygosity (0.554 in New Zealand White and 0.556 in APAU Black), expected heterozygosity (0.870 in New Zealand White and 0.875 in APAU Black), Polymorphic Information Content (0.856 in New Zealand White and 0.862 in APAU Black) and the mean effective number of alleles (8.629 in New Zealand White and 8.876 in APAU Black) of these two genetic groups were high. Out of the 12 amplified loci 7 loci deviated significantly from Hardy - Weinberg equilibrium in New Zealand White and 5 loci deviated in case of APAU Black which may be due to selection followed. The mean F_{IS}, F_{IT} and F_{ST} values over all the population were found to be 0.377, 0.402 and 0.040, respectively. The results suggested that the 12 amplified rabbit specific microsatellite loci were effective markers for analysis of genetic relationships among rabbit populations.

Keywords: Genetic diversity, microsatellite markers, New Zealand White rabbits, APAU Black rabbits

Organized promotion of rabbit production in India took place during the late seventies by the import of rabbits from the UK and the former USSR, by the Central Sheep and Wool Research Institute of the Indian Council of Agricultural Research. Of late, rabbit rearing has been attracting Indian farmers. The ever growing demand for animal proteins in developing countries like India helps the rabbit industry grow further and further. Though it has not made an impact in the meat industry in India, there is tremendous scope for popularizing rabbit meat to meet the increasing future demands.

For genetic improvement of any livestock, one needs to understand their genetic architecture which enables formulation of appropriate breeding strategies. Use of DNA markers to define the genetic makeup (genotype) of an animal is a powerful aid to animal breeding. Recent advances in molecular techniques for genetic characterization of livestock involving molecular markers such as RFLP, Microsatellites and SNPs have made it easy to explore the genetics of livestock.

Availability of genetic information in rabbits is scanty. Molecular characterization of the available rabbit genetic resources in India is not yet attempted so far. The molecular characterization forms the basis of genetic conservation of any species.

MATERIALS AND METHODS

Experimental animals and Microsatellite Primers

The present investigation was undertaken on 20 unrelated rabbits belonging to New Zealand White (10) and synthetic

variety APAU Black (10) rabbits which are maintained at Rabbit Research Centre, College of Veterinary Science, Rajendranagar, Hyderabad.

Approximately 4-5 ml blood was collected aseptically from each rabbit into tubes containing anticoagulant. Twenty microsatellite markers of which 12 rabbit specific [SAT02, SAT03, SAT04, SAT05, SAT07, SAT08, SAT12, SAT13, SAT16 (Mougel *et al.* 1997); SOL30 (Rico *et al.* 1994): SOL33, SOL44 (Surridge *et al.* 1997)] and 8 cross species [BM3205 (Bishop *et al.* 1994); ETH225 (Steffen *et al.* 1993); ILSTS005 (Brezinsky *et al.* 1993); ILSTS011 (Paiva *et al.* 2005); ILSTS017, ILSTS019, ILSTS033 (Kemp *et al.* 1995); TGLA227 (Ihara *et al.* 2004)] primers were used.

Genomic DNA extraction and PCR amplification of microsatellite loci

Genomic DNA was isolated from collected blood following standard Phenol-Chloroform method (Sambrook and Russel, 2001). UV spectrophotometer was used for estimation of the quantity of genomic DNA and the quality was estimated by electrophoresis of the isolated genomic DNA on 0.8 % agarose gels.

Selected 20 microsatellite loci (12 rabbit specific, 8 inter species) were amplified by Polymerase Chain Reaction (PCR) in 12.5 µl reaction mixture containing 1 µl (100 ng/µl) of Genomic DNA, 1.25 µl of 10X Taq Buffer, 0.25 µl dNTPs (10 mM/µl), 0.75µl of Forward- Primer (100 pM/µl), 0.75 µl of Reverse-Primer (100 pM/µl), 0.75 µl of $MgCl_2$ (25 mM/ µl), 0.5 U of Taq Polymerase (1 unit/ µl) and 7.25 µl of Autoclaved MilliQ water was added to make up the final volume. PCR conditions were: Initial denaturation at 95ºC for 5 min, followed by 34 cycles of 1 min at 94ºC, 30 sec at optimal annealing temperature, 30 sec at 72ºC and final extension step at 72ºC for 5 min. Amplification products along with 50 bp DNA ladder (for scaling) were resolved on 8% polyacrylamide gel and silver staining was used for visualising. Genotyping was conducted.

STATISTICAL ANALYSIS

$$\text{Expected heterozygosity} = 1 - \Sigma P_i^2$$

Where, P_i = frequency of i^{th} allele

The Polymorphism Information Content (PIC) was calculated by using the formula (Nei, 1978), as:

$$PIC = 1 - \sum_{i=1}^{k} P_{i^2} - \sum_{i=1}^{k-1} . \sum_{j=1}^{k} 2P_{i^2} P_{j^2}$$

Where,

P_i= frequency of i^{th} allele

P_j = frequency of j^{th} allele

-AZi and j = number of alleles

Coefficient of inbreeding (F_{IS}) is computed by the formula given by Hartl (2000) as,

$$F_{IS} = \frac{H_e - H_o}{H_e}$$

Where,

H_e is the expected heterozygosity within random mating subpopulations

H_o is the observed heterozygosity per individual within subpopulations

Fixation index (F_{ST})

$$F_{ST} = \frac{H_t - H_e}{H_e}$$

Where,

H_t is the expected heterozygosity in random mating total population

H_e is the expected heterozygosity within random mating subpopulations

Overall fixation index (F_{IT})

$$F_{IT} = \frac{H_t - H_o}{H_o}$$

Where,

H_t is the expected heterozygosity in random mating total population

H_o is the observed heterozygosity per individual within subpopulations

Test for Hardy-Weinberg equilibrium

The deviation of the population from Hardy-Weinberg equilibrium is an indication of intensity of external factors and this was tested by chi square test using the formula (Snedecor and Cochran, 1989)

$$x^2 = \sum_{i=1}^{k}\left[\frac{(O-E)^2}{E}\right]$$

Where,

O = Observed frequency

E = Expected frequency

k = Number of genotypes

RESULTS AND DISCUSSION

Mean number of alleles, effective number of alleles,

Table 1: Sample size (N), Mean number of alleles (N_a), Effective number of alleles (N_e), Observed heterozygosity (H_o), Expected heterozygosity (H_e) and Unbiased Expected heterozygosity (uH_e), Polymorphism Information content (PIC), Fixation Indices (F_{IS}) and Outcrossing rates at various microsatellite loci studied

Locus	N	Na	Ne	Ho	He	uHe	PIC	F_{IS}
SAT02	**9**	**11**	**10.125**	**0.556**	**0.901**	**0.954**	**0.893**	**0.384**
	10	15	13.333	0.900	0.925	0.974	0.920	0.027
SAT03	**9**	**8**	**6.231**	**0.444**	**0.840**	**0.889**	**0.819**	**0.471**
	9	11	9.529	0.333	0.895	0.948	0.885	0.628
SAT04	**10**	**12**	**10.526**	**0.800**	**0.905**	**0.953**	**0.897**	**0.116**
	10	14	40.526	0.600	0.905	0.953	0.898	0.337
SAT05	**9**	**10**	**9.000**	**0.556**	**0.889**	**0.941**	**0.878**	**0.375**
	10	9	6.061	0.300	0.835	0.879	0.816	0.641
SAT07	**10**	**14**	**11.765**	**0.900**	**0.915**	**0.963**	**0.908**	**0.016**
	9	10	8.100	0.778	0.877	0.928	0.864	0.113
SAT08	**10**	**10**	**7.407**	**0.400**	**0.865**	**0.911**	**0.851**	**0.538**
	10	10	7.407	0.300	0.865	0.911	0.851	0.653
SAT12	**10**	**7**	**5.000**	**0.200**	**0.800**	**0.842**	**0.773**	**0.750**
	10	9	6.987	0.400	0.855	0.900	0.839	0.532
SAT13	**10**	**14**	**11.765**	**1.000**	**0.915**	**0.963**	**0.908**	**-0.093**
	9	12	10.800	0.667	0.907	0.961	0.899	0.265
SAT16	**10**	**5**	**3.846**	**0.000**	**0.740**	**0.779**	**0.701**	**1.000**
	10	5	4.167	0.000	0.760	0.800	0.720	1.000
SOL30	**10**	**10**	**7.692**	**0.300**	**0.870**	**0.916**	**0.856**	**0.655**
	10	9	6.667	0.400	0.850	0.895	0.833	0.529
SOL33	**10**	**12**	**11.111**	**0.500**	**0.910**	**0.958**	**0.903**	**0.451**
	10	13	10.526	1.000	0.905	0.953	0.897	-0.105
SOL44	**10**	**11**	**9.091**	**1.000**	**0.890**	**0.937**	**0.880**	**-0.124**
	10	16	12.500	1.000	0.920	0.968	0.915	-0.087
Mean	**9.75**	**10.333**	**8.629**	**0.554**	**0.869**	**0.917**	**0.856**	**0.378**
	9.75	11.083	8.876	0.556	0.875	0.922	0.862	0.378

Note: The **Bold numbers indicates** the values for **New Zealand White** and light numbers for APAU Black animals

Shannon's information index, observed heterozygosity, expected and unbiased expected heterozygosity, polymorphism information content, fixation indices and outcrossing rates obtained in the present study were detailed in the Table 1. In comparison with the DNA concentration 2.28 µg/µl that was isolated from whole blood of mice by Hofstetter *et al.* (1997), and the DNA concentration 1.336 µg/µl that was isolated by phenol chloroform method from the whole blood of mammals by Al-azawy (2011), the overall mean concentration of isolated DNA from the both populations in the present study was slightly less (1.065 µg/µl).

Table 2: F-Statistics over the two populations for each locus

Locus	F_{IS}	F_{IT}	F_{ST}
SAT02	0.203	0.223	0.026
SAT03	0.552	0.571	0.044
SAT04	0.227	0.256	0.039
SAT05	0.504	0.529	0.052
SAT07	0.064	0.100	0.039
SAT08	0.595	0.611	0.038
SAT12	0.637	0.648	0.029
SAT13	0.085	0.118	0.035
SAT16	1.000	1.000	0.038
SOL30	0.593	0.614	0.052
SOL33	0.174	0.208	0.042
SOL44	-0.105	-0.055	0.045
Mean	0.377	0.402	0.040

Out of 20 microsatellites 12 loci which are rabbit specific loci amplified successfully and no positive results were obtained for inter species loci revealing that the primers designed for cattle, buffaloes, sheep and goats utilized in the present study are not amplifiable in the New Zealand White and APAU Black rabbits. In New Zealand White population a total of 124 and in APAU Black population a total of 133 alleles were amplified across the 12 rabbit specific loci. This indicated more genetic variation at these loci in APAU Black, which is a synthetic breed, when compared to the New Zealand White, which is a pure breed.

For estimation of genetic diversity between breeds or genetic groups at least 5 different alleles per locus were required (FAO, 2004). In the present study the overall mean number of alleles recorded was 10.333 and 11.083 in New Zealand White and APAU black populations respectively. In both the populations, a minimum of 5 or more alleles per locus were observed in all amplified 12 loci, indicating that sufficient number alleles were amplified to estimate the genetic diversity among these two genetic groups.

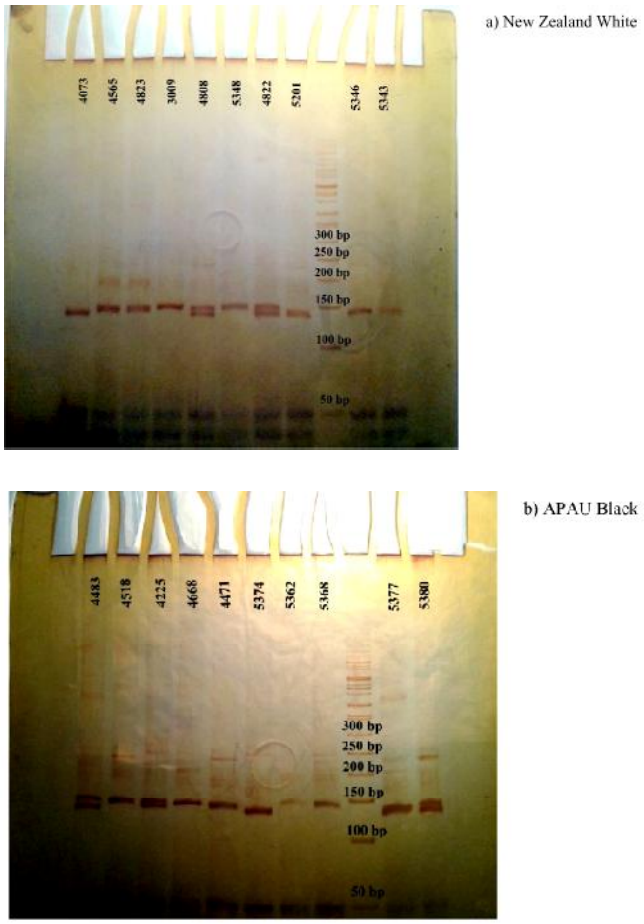

Fig. 1: PAGE image revealing polymorphism of microsatellite marker SAT 12

Genetic variability of a population is usually measured by the average heterozygosity per locus, while the gene differences between two populations may be measured by the genetic distance proposed by Nei and Roychoudhury (1972). The value of expected heterozygosity in New Zealand White population varied from 0.740 (SAT16) to 0.915 (SAT07) and in APAU black population the values ranged from 0.760 (SAT16) to 0.925 (SAT02).The results indicated that genetic diversity in both the populations

in the present study was high. This may be related to the breeding history and environment of each population. Observed heterozygosity ranged from 0.000 (SAT16) to 1.000 (SOL44) in both New Zealand White and APAU Black populations. Overall mean observed heterozygosity was 0.554 in New Zealand White population and 0.556 in APAU Black population.

According to Botstein *et al.* (1980) PIC index can be used to evaluate the level of gene variation. In this study the mean value of PIC was 0.862 and 0.856 in New Zealand White and APAU Black populations respectively. Each locus has the PIC value greater than 0.5 in both the populations, which indicated the presence of high diversity and highly informative nature of all the loci amplified.

The F_{IS} and F_{IT} may range from -1 to +1, however, the F_{ST} values were always positive. The overall mean values of $F_{IS,}$ F_{IT} and F_{ST} were 0.377, 0.402 and 0.040 respectively. Of the amplified 12 loci the F_{IS} values ranged from -0.105 (SOL44) to 1.000 (SAT16) and F_{IT} values ranged from -0.055 (SOL44) to 1.000 (SAT16). The negative values of F_{IS} (-0.105) and F_{IT} (-0.055) at SOL44 locus indicates occurrence of heterozygote genotypes at a proportion higher than the homozygous genotypes at that locus and the remaining loci showed positive values of F_{IS} indicating the heterozygote deficit in them among the two populations.

Mean F_{ST} value in the present study was 0.040 which ranged from 0.026 (SAT02) to 0.052 (SAT05 and SOL30). At the two loci SAT05 and SOL30 (F_{ST} value 0.052) moderate differentiation is observed between the two populations and all the remaining loci have the F_{ST} value less than 0.05 suggesting that differentiation did not exist between these two populations at these loci.

All the 12 loci (100%) amplified in this study were polymorphic in both the populations which correspond to the findings of Surridge *et al.* (1999) in European wild rabbits. While testing Hardy-Weinberg equilibrium in New Zealand White population 7 loci (SAT03, SAT05, SAT08, SAT12, SOL33 and SOL44) and in the APAU Black population 5 loci (SAT03, SAT05, SAT13, SOL30 and SOL44) were found to be deviated significantly from Hardy-Weinberg equilibrium.

CONCLUSION

The results suggested that the 8 inter species microsatellite loci were not amplified in the sample of rabbits taken under the present study and the 12 amplified rabbit specific microsatellite loci were effective markers for analysis of genetic relationships among rabbit populations and supported their suitability for genetic diversity studies and this can be used for designating priorities for their breeding.

REFERENCES

Al-azawy, A.F. 2011. A rapid, non-enzymatic method for genomic DNA extraction from whole blood and mammalian tissues. *Res. Opin. Anim. Vet. Sci.*, **1**(5): 279-283.

Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L., Hawkins, G.A. Hawkins, S.S. Toldo, R. Fries, M.D. Grosz, J. and Yoo, J. 1994. A genetic linkage map for cattle. *Genetics.*, **136**(2): 619-639.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **32**(3): 314.

Brezinsky, L., Kemp, S.J. and Teale, A.J. 1993. ILSTS005: a polymorphic bovine microsatellite. *Anim. Genet.*, **24**(1): 73.

FAO Standing Committee. 2004. Secondary guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD): Recommended Microsatellite markers.

Hartl, D.L. 2000. *A primer of population genetics*. 3rd Ed. Sinauer Associates, Inc., USA.

Hofstetter, J.R., Zhang, A., Mayeda, A.R., Guscar, T., Nurnberger Jr, J.I. and Lahiri, D.K. 1997. Genomic DNA from mice: a comparison of recovery methods and tissue sources. *Biochem. Mol. Med.*, **62**(2): 197-202.

Ihara, N., Takasuga, A., Mizoshita, K., Takeda, H., Sugimoto, M., Mizoguchi, Y., Hirano, T., Itoh, T., Watanabe, T., Reed, K.M., Snelling, W.M., Kappes, S.M., Beattie, C.W., Bennett, G.L. and Sugimoto, Y. 2004. A comprehensive genetic map of the cattle genome based on 3802 microsatellites. *Genome. Res.*, **14**(10a): 1987-1998.

Kemp, S. J., Hishida, O., Wambugu, J., Rink, A., Teale, A.J., Longeri, M.L., Ma, R.Z., Da, Y., Lewin, H.A. and Barendse, W. 1995. A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Anim. Genet.*, **26**(5): 299-306.

Nei, M. and Roychoudhury, A.K. 1972. Gene differences between Caucasian, Negro, and Japanese populations. *Science.*, **177**(4047): 434-436.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics.*, **89**(3): 583-590.

Paiva, S.R., Faria, D.A., Silvério, V.C., McManus, C., Egito, A.A., Dergam, J.A., Guimarães, S.E.F., Castro, S.R., Albuquerque, M.S.M. and Mariante, A.S. 2005. Genetic variability among Brazilian sheep using microsatellites. *Proceedings of The Role of biotechnology, Turin, March,* 5-7, 2005.

Rico, C., Rico, I., Webb, N., Smith, S., Bell, D. and Hewitt, G. 1994. Four polymorphic microsatellite loci for the European wild rabbit, Oryctolagus cuniculus. *Anim. Genet.*, **25**(5): 367-367.

Sambrook, J. and Russel, D.W. 2001. Molecular Cloning, Animal Laboratory manual. Cold Spring Harbor Laboratory press, Cold spring Harbor, New York.

Snedecor and Cochran. 1989. 'Statistical methods'. Oxford and IBH publishing Co; New Delhi.

Steffen, P., Eggen, A., Stranzinger, G., Fries, R., Dietz, A.B. and Womack, J.E. 1993. Isolation and mapping of polymorphic microsatellites in cattle. *Anim. Genet.*, **24**(2): 121-124.

Surridge, A.K., Bell, D.J., Ibrahim, K.M. and Hewitt, G.M. 1999. Population structure and genetic variation of European wild rabbits (Oryctolagus cuniculus) in East Anglia. *Heredity.*, **82**(5): 479-487.

Surridge, A.K., Bell, D.J., Rico, C. and Hewitt, G.M. 1997. Polymorphic microsatellite loci in the European rabbit (Oryctolagus cuniculus) are also amplified in other lagomorph species. *Anim. Genet.*, **28**(4): 302-305.