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Molecular Characterization of Black Bengal and Jamuna Pari Goat Breeds By RAPD Markers

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Abstract: This experiment was conducted in the Genetics, Breeding and Reproductive Biotechnology Laboratory under Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute (BLRI), Saver, Dhaka, Bangladesh. DNA was extracted from 14 goat breeds and the extracted DNA was observed by gel electrophoresis. Eight goat specific primers were synthesized by ASM-800 DNA synthesizer and screened in the study and all these primers were capable of priming polymorphic amplification pattern in both the breeds. Random amplification of polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) analysis was carried out using DNA samples of 14 black bengal goat and Jamuna pari goat breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. Data were analyzed by using a computer programe POPGENE (Version 1.31). Highest level of Nei's[1] gene diversity value (0.4898) was observed in BMS 1494 locus and the mean genetic diversity was obtained 0.3724 among the 14 goat breeds. The highest number of polymorphism obserbed in primer BM1818.The pair-wise genetic distance value ranged from 0.2500 to 1.000. Dendrogram based on Nei's[1] genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 14 goat breeds. Within Jamuna pari goat genetic similarity is low as well as black Bengal goat.

Key words: RAPD, Black Bengal goat, Jamuna Pari goat, Molecular Characterization, finger printings, genetic distance

INTRODUCTION

Black Bengal goat is the heritage and pride of Bangladesh. It is a dwarf breed found almost in all villages of Bangladesh. Like other domestic goat breeds in South Asia, Bengal goat believed to be derived from wild bezoar of Pasang (*Capra aegagrus*)[2] with infiltrated blood from markhor (*Capra falconeri*). Considering the geographical and cultural connection between south China and the Bengal area from old times across the South-Eastern off-shot of Tibetan plateau, Nozawa[3] suggested that Bengal goat inherit some genetic material from the South Chinese goats. Jamuna pari (Nubian type) is another important goat breed in Bangladesh.

A molecular marker is a DNA sequence which is readily detected and whose inheritance can easily be monitored. The use of molecular marker is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purpose. A marker must be polymorphic, that is, it must exist in different forms so that chromosome carrying the mutant gene can be distinguished from the chromosome with the normal gene by a marker it also carries[4].

Molecular markers are the molecules that could be used to trace a desired gene(s) in examined genotypes. In fact, a piece of DNA or a protein can be used as a marker. Earlier approaches that made selection of specific traits easier were based on the evaluation of morphological traits[5], isozymes[6] storage proteins like glutenins, gliadins, hordeins[7] etc. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment as morphological maekers.

Genetic diversity may be measured through genetic markers. These have been used to determine evolutionary relationship within and between species, genera or higher taxonomic categories[8]. However, breeders tend to concentrate on specific genotypes for determination of genetic diversity which combine traits of interest and may be used as progenitors in several breeding programmes in order to introduce agronomical important traits. In an attempt to solve the problem of maintaining pure breeds using the observed morphological characteristics that require a lot of time and effort, the use of molecular markers in maintaining goat breeds is more suitable and less time consuming. Moreover, molecular markers are important tools in

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tagging desirable loci underlying the traits which have breeding importance.

Estimation of genetic variation increasingly are being based upon information at the DNA level by various molecular markers such as, Randomly amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR) or Microsatellite etc. Among them, RAPD markers, generated by the polymerase chain reaction (PCR) is widely used since 1990's to assess infra-specific genetic variation at nuclear level[9,10].

RAPD is a PCR based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA. The technique was developed independently by two different laboratories[9,10] and called as RAPD and AP-PCR (Arbitrary Primed PCR) respectively. This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence.

The RAPD technology has provided a quick and efficient screen for DNA sequence-based polymorphisms at a very large number of loci. The major advantage is that no prior DNA sequence information is required. The vast range of potential primers that can be used gives the technique great diagnostic power. Reproducible RAPD bands can be found by a careful selection of primers, optimization of PCR conditions for the target species and replication to ensure that only the reproducible bands are scored.

RAPD analysis has been used extensively for various purposes which include identification and classification of accessions[11,12], identification of breeds[13], genetic diversity analysis[14-16] and predicting quantitative variation within germless[17]. RAPD markers have been successfully used for genetic variability analysis in a number of Animal species. These include cattle, goat, horse, buffalo, ostriches, chicken, broilers, quails, doves, pigs, emus etc.

The aim of this work is to evaluate genetic variation and relatedness of goat breeds by Random Amplified Polymorphic DNA (RAPD) technique. The study was conducted to determine the genetic variation between the breeds, to determine the genetic distance between two popular goat species, to select the suitable parent for crossing that can be used in hybrid production and to classify the goat breeds in Bangladesh.

MATERIALS AND METHODS

This experiment was conducted in the Genetics, Breeding and Reproductive Biotechnology Laboratory under Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute (BLRI), Saver, Dhaka, Bangladesh. In this chapter details of different materials used and methodologies followed for the study have been described.

We used RAPD markers for genetic characterization of those goat breeds.19 blood samples were collected from already selected pure Black Bengal goat and Jamuna pari goat from different areas. At least 5mL blood sample were collected in EDTA Vacutainer (Becton Dickinson) in the morning and after collection of the sample then kept into ice box. The samples were kept at –20°C upto the work. Before take the sample it is required to thaw.

Extraction of DNA from blood: Blood samples were collected in sterile polypropylene centrifuge tubes containing anticoagulant. The blood was gently mixed with anticoagulant and kept on ice to maintain low temperature in order to prevent cell lysis. Subsequently the blood samples were transported to the laboratory and stored at 4ºC until the isolation of genomic DNA. The genomic DNA was isolated by Roe *et al*.[18]. The isolated DNA was checked by mini-gel method[19].

Preparation of agarose gel: At first, 1.5g agarose powder (Invitrogen™ life technologies) was taken in a 500 mL Erlenmeyer flask containing 7.5mL electrophoretic buffer (20X TAE buffer) and 142.5mL distilled water The flask was enclosed with aluminum foil. Then the agarose was dissolved using microwave oven with occasional swirling until complete disappearance of agarose particles. A agarose solution was cooled to about 50°C. 25µL (5 mg mL^{-1}) ethidium bromide was added and mixed well by gentle shaking. The molten gel was poured immediately on to a clean gel bed (18x13x0.5 cm3 in size) and comb was inserted. Air bubbles were removed by pushing away to the side using a disposable tip. After one hour, gel became completely cooled at room temperature and solidified. The comb was removed gently and kept aside.

Primer selection: Eight goat specific primers were synthesized by ASM-800 DNA synthesizer and screened on a sub sample of two randomly chosen individuals from two different breeds to evaluate their suitability for amplification of the DNA sequences, which could be scored accurately. Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing and potential for population discrimination. The details of the primers are given in Table 1. A final subset of four primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis.

PCR amplification and agarose gel electrophoresis: The amplification conditions were based on Williams *et al*.[10] with some modifications. PCR reactions were performed on each DNA sample in a 10 µL reaction mix containing 2.5

Table1: Parameters of the Operon random primers

Primer Code	Primer sequence 5′-3′	GC content (%)
BM1818	R**- AGTGCTTTCAAGGTCCATGC	50
BMC1222	F*- CCAATTTTGCAGATAAGAAAACA	35
BMC1222	R- CCTGAGTGTTCCTCCTGAGT	55
BMS357	F-TCCAAACAAGTCTTCTCTATTTACC	45
BMS1494	R-AATGGATGACTCCTGCATGG	45
ILSTS005	R-TGTTCTGTGAGTTTGTAAGC	40
ILSTS17	F-GTCCCTAAAATCGAAATGCC	45
ILSTS44	F-AGTCACCCAAAAGTAACTGG	45

* Forward direction, ** Reverse direction

μL of 10X Ampli PCR buffer, 2 μL of 10 uM primer (Table 1), 1 μL of 250 uM dNTPs, 1 unit of Ampli *Taq* DNA polymerase (Invitrogen™ life technologies), $MgCl_2$ 1.5 μL and 2 μL genomic DNA and a suitable amount of sterile deionized water.

The PCR buffer, dNTPs, primer and DNA samples solutions were thawed from frozen stocks, mixed by vortexing and kept on ice. DNA template were pipetted first into PCR tubes compatible with the thermocycler used (0.2 mL). A pre-mix was then prepared in the course of the following order reaction buffer, dNTPs, DNA template and sterile distilled water. Taq polymerase was then added to the pre-mix. The pre-mix was then mixed up well and aliquoted into the tubes that already containing primer. The tubes were then sealed and placed in a thermocycle and the eyeing was started immediately.

DNA amplification was performed by thermal cycler (Mastercycle Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3minutes followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 38°C and elongation or extension of the primer at 72°C for 2 minutes. After the last cycle, a final step of 10 minutes incubation at 72°C was done. After completion of cycling program, reactions were held at 4°C.

PCR products from each sample were confirmed by running 2% agarose gel containing 5 μL ethidium bromide in IX TAE buffer at 70V, 300mA and 300W for 1.30 hrs. Loading dye (2.5 μL) was added to the PCR products and loaded in the wells. Two molecular weight markers DNA were also loaded on either side of the gel.Gels were stained with ethidium bromide as the solution of 25μL per 100 mL double distilled water. Place in staining solution for 45 minutes.

Detection was carried under UV light with Polaroid camera for capturing photograph. Each primer yielded a wide array of strong and weak bands.

Data analysis: RAPDs were scored according to the presence (1) or absence (0) of polymorphic DNA fragments. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. This was used for estimating polymorphic loci, Nei's[1] gene diversity, gene frequency, genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the populations using computer program POPGENE (Version 1.31)[20].

RESULTS AND DISCUSSION

RAPD amplification of polymorphic DNA-Polymerase Chain Reaction RAPD-PCR is a powerful molecular genetic technique for detection of' genetic variability and similarity in the different breeds/population .of, the livestock[21,22]. This technique is highly sensitive to minor alterations in the reaction conditions[23,24] hence; optimization of reaction conditions was carried out fur each of the primers by varying certain components of PCR. Cycling conditions were also optimized to get the good and reproducible results.

Table 2: Estimation of genetic diversity among 14 populations

Locus	Sample Size	h*
BM1818	14	0.1327
BMC1222F	14	0.4082
BMC1222R	14	0.4592
BMS1494	14	0.4898
Mean	14	0.3724
St. Dev		0.1634

* h = Nei's[1] gene diversity

Table 3: Estimation of gene frequency

Allele \ Locus	BM1818	BMC1222F	BMC1222R	BMS1494
Allele 0	0.9286	0.7143	0.6429	0.5714
Allele 1	0.0714	0.2857	0.3571	0.4286

Screening of RAPD primers: Eight random primers were used in representative 14 samples of Black Bengal Goat and Jamuna pari goat breeds in the study of amplification patterns. All of 8 primers were capable of exhibiting polymorphic amplification patterns and hence, they were used in the subsequent analysis with more number of DNA samples of the animals of these two breeds. Out of 8 primers 4 primers produced higher numbers of polymorphisms. Reproducibility of primer also checked and similar type of polymorphisms were obtained.

RAPD fingerprints: The individual samples from Black Bengal goats and Jamuna pari goat breeds were analysed with 8 primers screened in the study and all these primers were capable of priming polymorphic amplification pattern in both the breeds. Only unambiguous, reproducible and scorable polymorphic fragments were taken into consideration for analysis. The amplification patterns of representative samples of Black Bengal goats and Jamuna pari goat breeds with different primers. The primers BM1818, BMC1222 Forward, BMC1222 Reverse and BMS1494 Reverse produced total number of polymorphic bands as 15,12,32 and 9 respectively. But BMS357, ILSTS005, ILSTS17 and ILSTS44 showed less polymorphism.

Maximum number of polymorphic bands was 32 with primer BMC1222 Reverse and minimum with primer BMS1494.Average number of bands observed in 17.The highest number of polymorphic bands was found in Jamuna pari goat breeds with the 4 primers and it was 19 in number.

Table 4: Pair-wise mean genetic distance of 14 goat breeds

	B1	B2	B3	B4	B5	B6	B7	B8	B 9	J10	J11	J12	B13	B14
B1	****	1.0000	0.7500	0.5000	0.7500	1.0000	1.0000	0.2500	1.0000	0.7500	0.2500	1.0000	0.2500	0.5000
B2		****	0.7500	0.5000	0.7500	1.0000	1.0000	0.2500	1.0000	0.7500	0.2500	1.0000	0.2500	0.5000
B3			****	0.7500	1.0000	0.7500	0.7500	0.5000	0.7500	0.5000	0.5000	0.7500	0.0000	0.7500
B4				****	0.7500	0.5000	0.5000	0.7500	0.5000	0.7500	0.7500	0.5000	0.2500	0.5000
B5					****	0.7500	0.7500	0.5000	0.7500	0.5000	0.5000	0.7500	0.0000	0.7500
B6						****	1.0000	0.2500	1.0000	0.7500	0.2500	1.0000	0.2500	0.5000
B7							****	0.2500	1.0000	0.7500	0.2500	1.0000	0.2500	0.5000
B8								****	0.2500	0.5000	1.0000	0.2500	0.5000	0.7500
B9									****	0.7500	0.2500	1.0000	0.2500	0.5000
J10										****	0.5000	0.7500	0.5000	0.2500
J11											****	0.2500	0.5000	0.7500
J12												****	0.2500	0.5000
B13													****	0.2500
B14														****

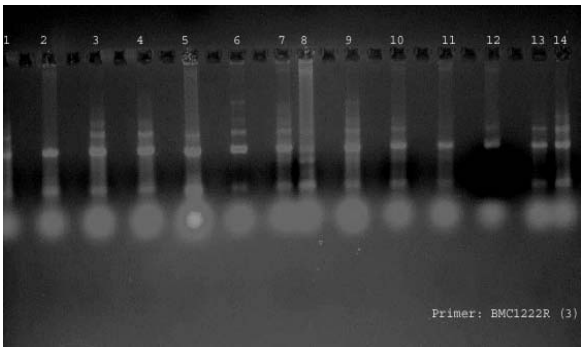

Fig. 1: RAPD profiles of fourteen goat breeds using primer BMC1222 Reverse. Lane 1-9 and 13 and 14 are Black Bengal goat and Lane 10-12 are Jamuna pari goat breeds

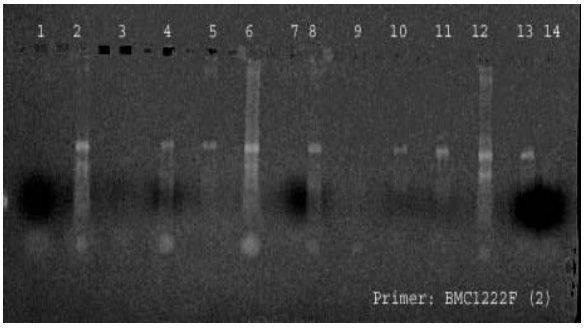

Fig. 2: RAPD profiles of fourteen goat breeds using primer BMC1222 Forward.Lane 1-9 and 13 and 14 are Black Bengal goat and Lane 10-12 are Jamuna pari goat breeds

Data were analyzed by using a computer programe POPGENE (Version 1.31)[20] and found dendrogram a UPGMA (Unweighted Pair Group Method of Arithmetic Means).

Gene diversity: Nei's[1] gene diversity values are shown in (Table 2). Highest level of Nei's[1] gene diversity value (0.4898) was observed in Bms1494 locus and the mean genetic diversity was obtained 0.3724 among the 14 goat breeds (Table 2).

Gene frequency: The highest level of gene frequency value (0.9286) was observed in allele 0 with the primer BM1818 and the lowest frequency value (0.0714) was obtained in allele 1 using same primer (Table 3). The RAPD method only score the homozygous and heterozygous dominant allele but unable to detect homozygous recessive one (H.S.Chawla).

Pair-wise genetic distance: The pair-wise mean genetic distance value ranged from (above diagonal) 0.2500 to 1.000.

Dendrogram without root: Dendrogram based on Nei's[1] genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 14 goat breeds. Within Jamuna pari goat genetic similarity is low as well as black Bengal goat. But genetic distance is high between the two breeds. All data were analyzed by a computer programme, POPGENE (Version 1.31) [20]. The UPGMA dendrogram (Fig. 1) shows that the 14 breeds divided in three main clusters. Cluster 1 consisted of B1609, B1853, B1952, B1969, B1990, J22 and J01. The 2nd cluster consisted of three breeds like B070104, B1861 and B1752 and cluster 3 consisted with B080302, B1648, B1972 and J08.Cluster 1 shows Black Bengal goat and Jamuna pari goat breeds of same region. Cluster 2 and 3 is concentrated with all black Bengal goat breeds.

Dendrogram with root: Dendrogram with root means to show the ancestors of the progenies. In the present study 14 goat breeds were studied for their genetic characterization J01, J08 and J22 are Jamuna pari goat breeds and B1609, B1853, B1861, B1648, B1752, B1952, B1969, B1972, B1990, B070104 and B080302 are black Bengal goat breeds. Cluster 1 consisted of B1609, B1853, B1952, B1969, B1990, J22 and J01 that mean those had a common ancestor. The 2nd cluster consisted of three breeds like B070104, B1861 and B1752 probably those had a common ancestor. And cluster 3 consisted with B080302, B1648, B1972 and

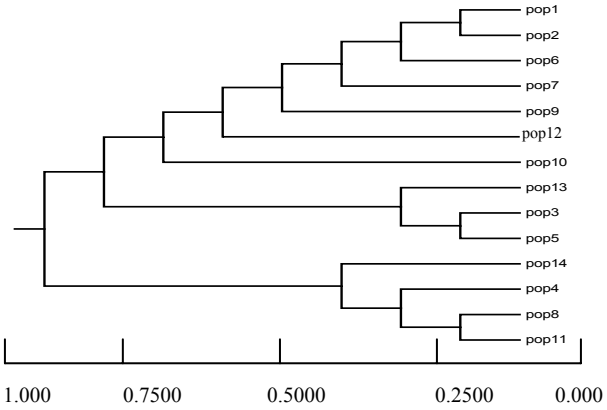

Fig. 3: UPGMA Dendrogram without root

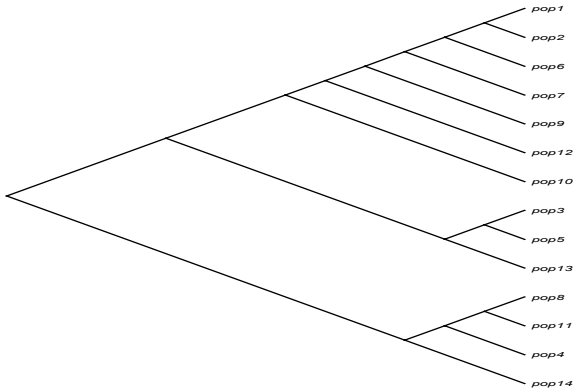

Fig. 4: UPGMA dendrogram with root

J08 may be originated from the similar root. Cluster 1,2 and 3 also shows a common root and this point means black Bengal goat breeds may have the similar root. Jamuna pari breeds J22 and J01 also show the similar origin, this may need further study.

CONCLUSION

It is concluded that the results of the present study indicated that using these markers genetic diversity within black Bengal goat breeds is low and genetic distance within Jamuna pari goat breeds also low. Genetic distance between black Bengal goat and Jamuna pari goat breeds is very high. In fact according to the report of Kantanen *et al*.[25], goat breeds have high degree of homogeneity. This RAPD work is the initial work in Bangladesh and has studied only with 14 goat breeds. It is suggested to study with more primers and large samples to analyze genetic characteristics between Black Bengal goat and Jamuna pari goat breeds.

REFERENCES

1. Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. United States of America, 70: 3321-3323.
2. Herre, W. and M. Rohrs, 1973. Haustiere-zoologich gesehen. (Domestic animal- zoological aspects). Stuttgart, Garmen Federal Republic; B, Fischer.
3. Nozawa, K., 1991. Domestication and History of goats. In: Genetic Resources of Pig, Sheep and Goat. World Animal Science B8. (Ed., K. Maijala). Elsevier Science Publishers. B.V. Amsterdam, The Netherlands, pp: 391-404.
4. Chawla, H.S., 2002. Introduction to Plant Biotechnology. Oxford and IBH Publishing Co. Pvt. Ltd., Calcutta and New Delhi, India.
5. Staub, J.E., F.C. Serquen and M. Gupta, 1996. Genetic markers, map construction and their application in plant breeding. Horticult. Sci., 31: 729-741.
6. Stuber, C.W. and K.R. Khanna, 1991. Isozyme Markers and their Significance in Crop Improvement. Biochemical Aspects of Crop Improvement. CRC Pres, Boca Raton, USA, pp: 59-77.
7. Vapa, L. and D. Radovic, 1998. Genetics and molecular biology of Barley hordeins. Cereal Res. Commun., 26: 31-38.
8. Paterson, A.H., S.D. Tanksley and M.E. Sorrells, 1991. DNA markers in plant improvement. Advan. Agron., 46: 39-90.
9. Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.
10. Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic maekers. Nucleic Acids Res., 18: 6531-6535.
11. Fukuoka, S., K. Hosaka and O. Kamijima, 1992.Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. Jap. J. Genet., 67: 243-252.
12. Virk, P.S., H.J. Newbury, M.T. Jackson and B.V.L. Ford, 1995. The identification ofduplicate accessions within a rice germplasm collection using RAPD analysis. Theor. Appl. Genet., 90: 1049-1055.
13. Qian, Q., H. Chen, Z.X. Sun and L.H. Zhu,1996. The study on determining true and false hybrid rice II You 63 using RAPD molecular markers. Chin. J. Rice Sci., 10: 241-242.

14. Yu, L.X. and H.T. Nguyen, 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet., 87: 668-672.
15. Mackill, D.J., 1995. Classifying japonica rice cultivars with RAPD markers. Crop Sci., 35: 889-894.
16. Cao, D. and J.H. Oard, 1997. Pedigree and RAPD-based DNA analysis commercial U.S. rice cultivars. Crop Sci., 37: 1630-1635.
17. Virk, P.S., B.V.L. Fond, M.T. Jackson, H.S. Pooni, T.P. Clemeno and H.J. Newbury, 1996. Predicting quantitative variation within rice germplasm using molecular markers. Heredity, 76: 296-304.
18. Roe, B.A., J.S. Crabtree and A.S. Khan, 1996. DNA isolation and sequencig: Essential Techniques.
19. Sambrook. J., Maniatis. T. and Fritsch E.F.1984. Molecular Cloning. A Laboratory Manual. Sring Harbor laboratory, USA.
20. Yeh, F.C., R.C. Yang, T.B. J.Boyle, Z.H. Ye and J.X. Mao, 1999. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Bitechnology Centre, university of Alberta, Canada. (http:// www. ualbert.ca/~fyeh).
21. Kemp, S.J. and A.J. Teale, 1992. Random amplified DNA polymorphisms (RAPDs) and pooled DNA bovine genetic study. Anim. Genet., 23: 62.
22. Cushwa, W.T., K.G. Dodds, A.M. Crawferd and J.F. Medrano, 1996. Identification and genetic mapping of random amplified polymorphic DNA (RAPD) markers to the sheep genome. Mann. Genome, 7: 550-585.
23. Yu, K.F. and K.P. Pauls, 1992. Optimization of the PCR programmme for RAPD analysis. Nucleic Acid Res., 20: 1606.
24. Bowditch, B.M., D.G. Albright, J.G.K. Willians and M.J. Braun, 1993. Use of randomly amplified polymorphic DNA marker in comparative genome studies. Methods in Enzymol., 224: 294-309.
25. Kantanen, J., J. Vilkki, K. Elo and A. Maki-Tanila, 1995. Random amplified polymorphic DNA in cattle and sheep: Application for detecting genetic variation. Anim. Genet., 25: 315-320.