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Microsatellite Marker Based DNA Fingerprinting for Parentage Verification in Goat Breeds of Kashmir

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A B S T R A C T

Bhakerwal and Pashmina are local Kashmiri goat breeds usually reared by nomadic people and people of Changthang region of Ladakh respectively. They play promising role in alleviating poverty of this far flung region of the country. Adequate breeding and research have been done in these animals to increase their population and quality but parentage analysis and pedigrees record keeping has been a limitation since long time. Keeping this in view the present study was conducted to validate a panel of 8 microsatellite markers in 10 families of Bhakerwal and Pashmina breeds so that this limitation can be overcome and breeding and research of these breeds could be carried prudently in future.

Introduction

Correct pedigree recording is essential for genetic improvement programs in livestock species. Pedigree errors of about 10% may lead to reductions in selection response of two to three percent in dairy cattle (Visscher *et al.*, 2002), while different studies have reported pedigree errors of up to 23% in cattle in several countries (Jiménez-Gamero *et al.*, 2006). Goats are primarily farmed under extensive production systems and mating systems used by breeders taking part in the Stock Improvement Schemes primarily include group mating and flock mating. These mating practices limit accurate pedigree recording. Other factors that contribute to potential errors in identification of the parents include the use of large paddocks in extensive production systems (Bolormaa *et al.*, 2008). So, correct parentage among breeding stock is a pre-requisite for an efficient breeding programme. Besides, parentage analysis finds its importance in paternity disputes in

animals. The markers revealing variation at DNA sequence level are referred as DNA markers which can be Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNP), Variable Number Tandem Repeats (VNTRs) and Simple Sequence Repeats (SSRs).

Microsatellite markers are used nowadays for DNA fingerprinting which are DNA sequences of one to six units repeated in tandem. Microsatellite markers are ideal for parentage verification because they are randomly distributed throughout the genome, commonly occurring in noncoding regions, and are selectively neutral. Microsatellite alleles also show codominant inheritance, making them relatively easy to score directly. Microsatellites can be identified by PCR amplification followed by amplicon scoring.

Amplicon scoring can be performed by amplicon sequencing, radiolabelled primers (probes), fluorescent dye labelled primers, metaphor gel electrophoresis or by denaturation or native PAGE. Using any of the above mentioned method the exact size of amplicon of different alleles is obtained which can be used for parentage verification.

Materials and Methods

The present investigation was undertaken on ten families of Bhakerwal and Pashmina goats maintained at Mountain Research Centre of Sheep and Goat, F.V.Sc and A.H, Shuhama, Alasteng. Genomic DNA was isolated from the blood samples using the standard Phenol-Chloroform extraction protocol of Sambrook and Russell (2001) Microsatellite markers as listed in Table 1 were used for amplification of DNA samples of the germplasm under investigation.

PCR was carried out in a final reaction volume of 20 µl in 200 µl thin walled sterilized PCR tubes. All conditions were same for 8 microsatellite markers except the annealing temperature. Annealing temperature for ILSTS-019, ILSTS-022, ILSTS-030, MAF214, oarJMP58, BM4301 and INRA081 was 58°C, whereas annealing temperature for oarAE129 was 60°C. The PCR amplicons were further separated on 3% metaphor agarose gel electrophoresis to determine allelic variation at each locus. For statistical analysis Mean number of alleles, Polymorphism Information Content (PIC), Heterozygosity (Observed and Expected) were calculated using GenAlEx software. These parameters were used for determination of non-exclusion probability utilising the software Cervus 3.0.

Results and Discussion

Total numbers of alleles observed in present study were found to be 50. Maximum number of alleles per marker observed across the populations was 9 for BM4301. The mean numbers of alleles observed were 6.20. This mean explains high level of polymorphism of the studied microsatellites. Nearly similar observation were reported for Italian goat breeds (NA=6.5; Agha *et al.*, 2008). However, average values observed in present study was lower than the Croatian spotted breed (NA=8.1; Jelena *et al.*, 2011), the average value of seven Indian goat breeds (NA=8.1-9.7; Rout *et al.*, 2008). The average PIC value was 0.7606. Non-exclusion probability determines the extent to which a particular individual could be considered parent and cannot be eliminated. Non-exclusion could be for one parent or other parent or together for a parent pair. The Combined Non-exclusion probability for first parent was found to be 0.998, for second parent it was found to be 0.9999 and for parent pair it was found to be 0.99999.

Table.1 Primer sequences for different microsatellites used for DNA fingerprinting

S. No	**Microsatellite**	**Markers Primer sequences (5′-3′)**	**Reference**
1	BM4301	**F:** CCACATGTCTCAAAGCAACG **R:** GGAAAAGGGTCTTTGTTGAGC	Stone *et al.,* (1995)
2	INRA081	**F:** CGGCTCACGGTCTCTATCGG **R:**GCGAACCCAAGAATCAGACTC	Ihara*et al.*,(2004)
3	MAF214	**F**:AATGCAGGAGATCTGAGGCAGG **R:** CCACTCCTCCTGAGAATATAACATG	Buchanan *et al.,* (1992)
4	ILSTS-022	**F:** TCTATACACATGTGCTGTGC **R:** CTTAGGGGTGAAGTGACACG	Kemp *et al.,* (1995)
5	oarJMP58	**F:**GAAGTCATTGAGGGGTCGCTAACC **R:**CTTCATGTTCACAGGACTTTCTCTG	Lumsden *et al.,* (1996)
6	ILSTS-019	**F:** AAGGGACCTCATGTAGAAGC **R:** ACTTTTGGACCCTGTAGTGC	Kemp *et al.,* (1993)
7	ILSTS-030	**F**: CTGCAGTTCTGCATATG **R**: CTTAGACAACAGGGGTTTGG	Ma *etal.,* (1996)
8	oarAE-129	**F:**GATCACAAAAAGTTGGATACAACCGTGG **R:** TCATGCACTTAAGTATGTAGGATGCTG	Penty *et al.,* (1993)

Table.2 A summary of the non-exclusion probability of different markers for parentage verification

S. No.	**Locus**	**PNE-1**	**PNE-2**	**PNE-PP**
1.	BM4301	0.431	0.273	0.111
2.	ILSTS 019	0.556	0.379	0.199
3.	ILSTS022	0.716	0.546	0.372
4.	ILSTS030	0.668	0.491	0.304
5.	INRA081	0.536	0.361	0.185
6.	MAF214	0.580	0.402	0.221
7.	oarJMP58	0.627	0.448	0.262
8.	oarAE129	0.506	0.334	0.161
CNE		**0.9998**	**0.9999**	**0.9999**

PNE1 – Probability of Non-Exclusion for one candidate parent alone.
PNE2 – Probability of Non-Exclusion for one candidate parent and one known parent.
PNE-PP –Probability of Non-Exclusion when both parents are known.
CNE= Combined Non-Exclusion Probability.

This means that one parent could be taken as a parent with 0.998 probability, the other with 0.999 probability and parent pair could be determined using these 8 microsatellite markers with 0.9999 probability. Same was reported by Luikart *et al.,* (1999) by using 22 microsatellite markers in fluorescent multiplexes. These results suggest that the DNA typing method has high potential for parentage verification. The conclusions drawn are that 8 microsatellites were highly polymorphic and proved very useful for DNA fingerprinting in goats, the study can be extended to include more microsatellites and can be extended to other species of animals, the panel of microsatellite markers studied is hereby validated for checking and correcting the pedigree records of goats which is expected to increase the accuracy of selection and selection response leading to efficient genetic improvement.

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