

Genetic polymorphism in Jakhrana goats and their comparison with other Indian goat breeds through RAPD

AARTI SHARMA¹ and SAKET BHUSAN²

ICAR-Central Institute for Research on Goats, Makhdoom, Uttar Pradesh 281 122 India

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ABSTRACT

RAPD markers were used to estimate the genetic variability of Jakhrana breed with other extant goat breeds, and to evaluate genetic relationship between them. Adult females, 15 from each breed (Jakhrana, Barbari, Sirohi, Black Bengal and Jamunapari), were randomly selected. Polymorphic random primers OPM-12, OPM-18, OPM-14, OPA-01, OPM-05, OPP-08, OPP-02 were used for analysis. The estimates were based on band sharing proportion and based on the band frequency. When pooled over the primers, based on band sharing, Black Bengal (0.890) showed minimum while Jakhrana showed maximum (0.994) within-breed genetic similarity. Similar trend was observed based on band frequency where Black Bengal (0.800) showed minimum and Jakhrana showed maximum (0.890) within breed genetic similarity. For between-breed similarities, based on band sharing, genetic similarity was observed minimum between Jamunapari and Jakhrana (0.846) while maximum was observed between Sirohi and Jakhrana (0.994), based on band frequency, the minimum was observed between Jamunapari and Sirohi (0.985), while Black Bengal and Barbari showed maximum genetic similarity of 0.999. These results can be utilized to upgrade and improve the breeds which are genetically close through a common breed improvement program.

Key words: Genetic distance, Genetic similarity, Jakhrana, Polymorphism, RAPD

The goat, one of the most important livestock species in India, provides food and nutritional security to marginal farmers, small farmers and land less labourers. Goats originated in Asia and spread to other continents and inhabitant over all the climatic zones. India has 140.54 million goats which produce 4.59 million tonnes milk and 0.85 million tonnes meat. Not enough research has been done on conservation, sustainable use of their genetic diversity, breed characteristics, phylogenetic traits, and their production potential is far from enough compared with other livestock species. Indian goat breeds are diverse in their performance traits. Molecular characterization of different goat breeds has also become essential to establish the current status of goat breeds in different climatic zones of the country (Joshi *et al.* 2004). Study of genetic relation between and within different breed of goat is required for conservation and improvements through a common breed improvement program. Genetic markers provide useful information to wide range of comparative genetic research endeavors (Yu Qi *et al.* 2009). The RAPD may provide a highly polymorphic system of choice, capable of generating suitable information with regards to estimate the genetic

variability and to establish genetic relationship among the different populations. The RAPD markers have successfully been used for estimation of genetic variation and similarities between different breeds and species of livestock (Kemp and Teale 1994, Kantanean *et al.* 1995, AppaRao *et al.* 1996). Sharma *et al.* (2001) found that RAPD technique detects sufficient polymorphism within and between breeds. Anbarasan *et al.* (2001, 2002) worked successfully on genetic relatedness and genetic differentiation among many descript and non-descript Indian goats using randomly amplified polymorphic DNA (RAPD) markers. In the present study Jakhrana, Barabari, Sirohi, Jamunapari, and Black Bengal goat breeds were taken to identify their relation with each other at DNA level through RAPD markers.

MATERIALS AND METHODS

Fifteen animal of each breed of goat i.e. Jakhrana, Barbari, Sirohi, Jamunapari and Black Bengal were taken for the study. Blood (5 ml) from each animal was collected from jugular vein in vacutainer tubes containing EDTA as anticoagulant (Sambrook and Russel 2001). Genomic DNA was isolated using phenol-chloroform extraction method. The quantity of DNA was measured by UV spectrophotometer and quality of DNA was checked by agarose gel electrophoresis. Total 20 random primers having

*Part of Ph. D. Thesis of first author,

Present address: ¹Ph. D. Scholar (aartisharma.bt@rediffmail.com), ²Principal Scientist (skbcirg@gmail.com).

Table 1. Characteristics of the amplification profiles from the 20 random primers for different goat breeds

Primer	Number of bands			
	Total	Polymorphic	Max.	Min.
OPM-08	6	0	1700	290
OPM-09	3	0	1350	400
OPM-10	10	0	1660	305
OPM-12	6	4	1550	350
OPM-18	8	4	1800	250
OPM-14	3	3	2000	190
OPM-05	6	2	2100	310
OPA-01	5	4	1200	250
OPA-05	12	0	1500	400
OPA-10	7	0	1510	460
OPA-13	9	0	2450	210
OPA-15	12	0	2300	350
OPA-19	3	0	1510	360
OPA-18	6	0	920	400
OPA-20	3	0	2000	350
OPP-08	5	4	1400	320
OPP-02	4	2	3300	510
OPP-04	4	0	1500	405
OPP-17	6	0	660	210
OPB-07	6	0	900	380

GC content of 60–90 % were screened on pooled DNA samples of each breed. Polymorphic primers were used for the further analysis. The list of primers, their sequence and GC content is given in Table 1. PCR was carried out in a final volume 50 ml in following reaction components. 1 X PCR buffer, 1.5 mM MgCl₂, 10 mM dNTPs mix, 2.5 U Taq DNA polymerase, 20 pmol of each primer and 100 ng genomic DNA. The amplification was performed in thermal cycler with initial denaturation at 94⁰ for 2 min, followed by denaturation at 94⁰ for 1 min, annealing at 40⁰ for 1 min, extension at 72⁰ for 2 min for 40 cycles and final extension at 72⁰ for 5 min. Number of bands resolved on gel with different primers was recorded. Only distinct and prominent bands were scored. The RAPD patterns were scored for the presence or absence of amplicon and the data entered into a binary character matrix. If the band is present, it was designated as “1” and absence as “0”. This matrix was further used in estimating the genetic similarity as well as genetic distances within as well as between breeds based on band sharing and the band frequency proportion and statistical analysis was used for analyzing the PCR-RAPD data (Nei and Lei 1979, Lynch 1990).

RESULTS AND DISCUSSION

The characteristics of the amplification profiles from the 20 random primers for different goat breeds are presented in Table 1. The total number of bands amplified ranged from 3 to 12. While primer OPM -09, OPM-14, OPA-19

and OPA-20 amplified least number of band (i.e. 3 only), a maximum of 12 bands were amplified by primer OPA -05 and LOPA-15. The bands amplified from these primers also varied in their size ranges. The size difference between the maximum and minimum size of amplified band by a primer varied from 190 bp (OPM-14) to 3300 bp (OPP-02). Out of 20 random primers capable of generating distinct and repeatable RAPD profile, only 7 primers (35%) could detect polymorphism (Table 2) between the 5 goat breeds, while rest of the primers generated monomorphic RAPD pattern. Primer OPM-14 could detect polymorphism within the Sirohi, Barbari, Jamunapari and Black Bengal, however produced monomorphic RAPD profile within breed Jakhana. Similarly primer OPA-01 produced polymorphic RAPD profile within Sirohi, Black Bengal, Jakhana and Jamunapari breeds, but monomorphic RAPD profile within Barbari breeds. Primer OPP-02 showed polymorphic pattern to Sirohi, Jakhana, Jamunapari, and Barbari breeds, while monomorphic RAPD profile to Black Bengal breed. Other 4 primers i.e. OPM-12, OPP-08, OPM-05 and OPM-18 detected polymorphism within all the 5 goat breeds (Fig. 1). The earlier workers also observed that out of many tested primers only a few produced polymorphism (Chung *et al.* 1999, Guneren *et al.* 2010, Yadav *et al.* 2008., Wei *et al.* 2002, Anbarasan *et al.* 2001, CengizElmaci *et al.* 2007).

Within-breed genetic similarity: Within-breed genetic similarity as band sharing proportion (WS) and band frequency (WF) estimated from 7 polymorphic primers are presented in Tables 3 and 4, respectively. The magnitude of the Within-breed genetic similarity for these breeds differed with the primer used since marker genes were polymorphic in nature. When pooled over primers, lowest and highest Within-breed genetic similarity based on band sharing was 0.890 and 0.994 for Black Bengal and Jakhana breeds, respectively. Similarly, when pooled over primers, lowest and highest Within-breed genetic similarity based on band frequency was 0.800 and 0.930 for Black Bengal and Jakhana breeds, respectively. Similar results were reported by Anbarasan *et al.* (2002) Within-breed genetic similarity (0.84–0.92) within 4 goat breeds, viz. Black Bengal (BB), Barbari (B) and Chegu (C) and one local non-descript (ND) goats.

Between-breed genetic similarity: The Between-breed genetic similarity estimates from band sharing (BS) and

Table 2. List of random primers screened among the goat population for polymorphism detection

Primer name	Primer sequence	G+C content (%)
OPM-12	GGGACGTTGG	70
OPM-18	CACCATCCGT	60
OPM-14	AGGGTCGTTC	60
OPA-01	CAGGCCCTTC	70
OPM-05	GGGAACGTGT	60
OPP-08	ACATCGGCCA	60
OPP-02	TCGGCACGCA	70

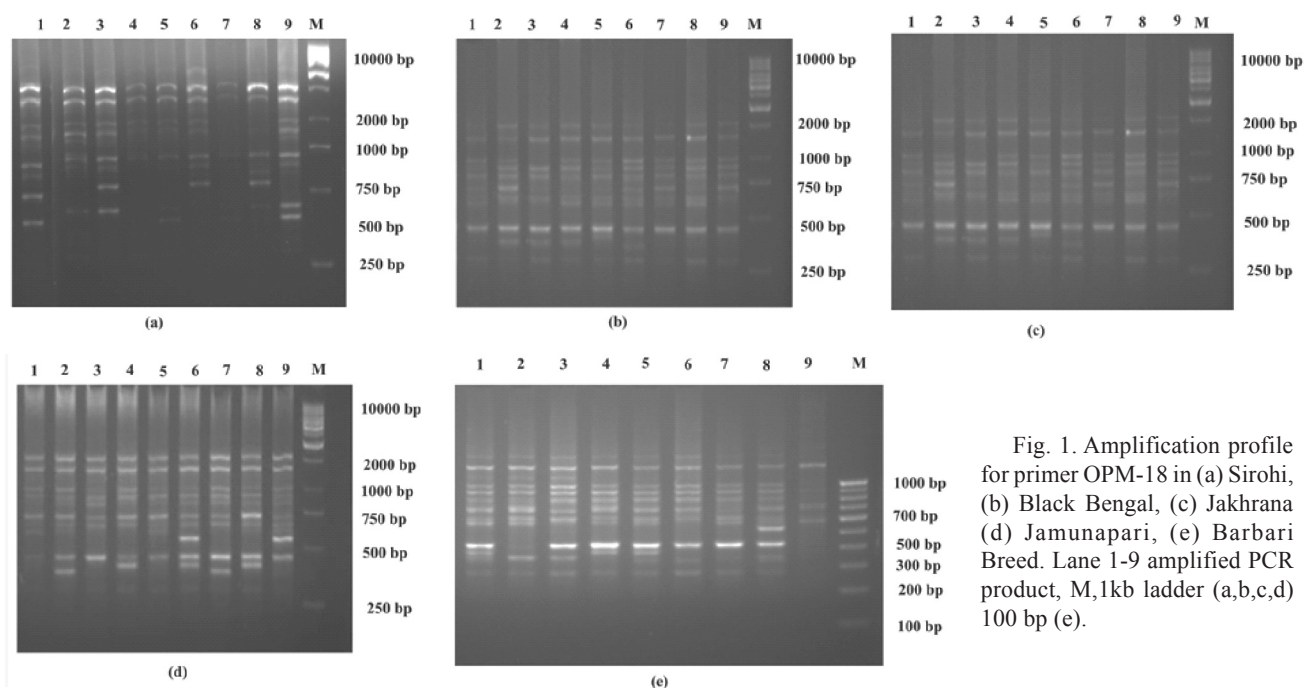


Fig. 1. Amplification profile for primer OPM-18 in (a) Sirohi, (b) Black Bengal, (c) Jakhrana (d) Jamunapari, (e) Barbari Breed. Lane 1-9 amplified PCR product, M, 1kb ladder (a,b,c,d) 100 bp (e).

Table 3. Within-breed genetic similarity as estimated from band sharing (BS) in different goat breeds from RAPD analysis with seven polymorphic primers

Breeds	Primers							Pooled over primers
	OPM-12	OPM-18	OPM-14	OPA-01	OPM-05	OPP-08	OPP-02	
Sirohi	0.850	0.950	0.970	0.800	0.950	0.850	0.900	0.907
Black Bengal	0.880	0.880	1.000	0.928	0.920	1.000	1.000	0.890
Jakhrana	0.830	0.880	0.930	0.790	0.796	0.836	0.880	0.994
Jamunapari	0.860	1.000	1.000	0.930	0.900	0.920	1.000	0.920
Barbari	0.870	0.940	0.910	0.890	0.800	0.900	0.947	0.960

Table 4. Within-breed genetic similarity (WF) as estimated from band frequency in different goat breeds from RAPD analysis with seven polymorphic primers

Breeds	Primers							Pooled over primers
	OPM-12	OPM-18	OPM-14	OPA-01	OPM-05	OPP-08	OPP-02	
Sirohi	0.690	0.940	0.820	0.800	0.900	0.700	0.751	0.890
Black Bengal	0.850	0.856	1.000	0.856	0.850	1.000	0.850	0.800
Jakhrana	0.750	0.870	0.910	0.790	0.790	0.914	0.750	0.930
Jamunapari	0.810	1.000	1.000	1.000	0.928	0.920	0.815	0.890
Barbari	0.870	0.890	0.890	0.892	0.896	0.875	0.870	0.830

band frequency (BF) between the 5 goat breeds presented in above diagonal of Table 5 and 6 respectively. With the primer used, genetic similarity estimates differed in magnitude. However, when pooled over primers, the minimum Between-breed genetic similarity was observed lowest between Jamunapari and Jakhrana (0.946), while Sirohi and Jakhrana showed maximum genetic similarity of 0.994. Ghosh *et al.* (2001) also observed limited, but easily detectable DNA polymorphisms among different Indian goat breeds viz. Barbari, Beetal, Jamunapari,

Jakhrana. However, with pooled over primers, the minimum Between-Breed genetic similarity was observed between Jamunapari and Sirohi (0.985), while Black Bengal and Barbari showed maximum genetic similarity of 0.999.

Between-breed genetic distances: The Between-breed genetic distance estimates from band sharing (BS) and band frequency (BF) between the goat breeds are presented in below diagonal of Table 5 and 6, respectively. When pooled over primers, the minimum Between-breed genetic distance

Table 5. Between breed genetic similarities (above diagonal) and genetic distances (below diagonal) estimates based on band sharing (BS) among the different goat breeds

Primers	Breeds	Breeds				
		Sirohi	Black Bengal	Jakharana	Jamunapari	Barbari
OPM-12	Sirohi	-	0.941	0.934	0.948	0.983
	Black Bengal	0.09	-	0.952	0.981	0.963
	Jakharana	0.080	0.058	-	0.960	0.969
	Jamunapari	0.063	0.008	0.046	-	0.979
	Barbari	0.016	0.041	0.048	0.024	-
OPM-18	Sirohi	-	0.990	0.994	0.753	0.970
	Black Bengal	0.009	-	1.000	0.755	0.972
	Jakharana	0.004	0.018	-	0.720	0.973
	Jamunapari	0.290	0.032	0.349	-	0.790
	Barbari	0.012	3.032	0.030	0.030	-
OPM-14	Sirohi	-	0.911	0.885	0.971	0.976
	Black Bengal	0.094	-	0.967	0.855	0.980
	Jakharana	0.126	0.196	-	0.826	0.947
	Jamunapari	0.028	0.154	0.196	-	0.959
	Barbari	0.023	0.020	0.057	0.043	-
OPA-01	Sirohi	-	0.952	0.994	0.873	0.962
	Black Bengal	0.051	-	0.989	0.789	0.956
	Jakharana	0.004	0.009	-	0.878	0.989
	Jamunapari	0.153	0.256	0.148	-	0.910
	Barbari	0.042	0.047	0.000	0.232	-
OPM-05	Sirohi	-	0.940	0.932	0.942	0.980
	Black Bengal	0.051	-	0.951	0.979	0.960
	Jakharana	0.004	0.009	-	0.959	0.960
	Jamunapari	0.152	0.255	0.147	-	0.980
	Barbari	0.041	0.046	0.000	0.230	-
OPP-08	Sirohi	-	0.910	0.884	0.970	0.974
	Black Bengal	0.051	-	0.965	0.852	0.982
	Jakharana	0.004	0.009	-	0.825	0.945
	Jamunapari	0.153	0.256	0.148	-	0.958
	Barbari	0.042	0.047	0.000	0.232	-
OPP-02	Sirohi	-	0.860	0.810	0.950	1.000
	Black Bengal	0.009	-	0.775	0.850	1.000
	Jakharana	0.004	0.018	-	0.890	0.899
	Jamunapari	0.290	0.030	0.348	-	0.890
	Barbari	0.012	0.030	0.030	0.030	-
Pooled over primers	Sirohi	-	0.952	0.994	0.884	0.978
	Black Bengal	0.057	-	0.984	0.852	0.966
	Jakharana	0.054	0.020	-	0.846	0.969
	Jamunapari	0.130	0.170	0.183	-	0.848
	Barbari	0.023	0.035	0.034	0.133	-

Table 6. Between breed genetic similarities (above diagonal) and genetic distances (below diagonal) estimates based on band sharing (BF) among the different goat breeds

Primers	Breeds	Breeds				
		Sirohi	Black Bengal	Jakharana	Jamunapari	Barbari
OPM-12	Sirohi	-	1.000	0.998	0.989	0.975
	Black Bengal	0.000	-	0.990	1.000	0.999
	Jakharana	0.002	0.000	-	0.998	0.990
	Jamunapari	0.011	0.000	0.002	-	0.999
	Barbari	0.025	0.001	0.010	0.010	-
OPM-18	Sirohi	-	0.989	0.996	0.999	0.994
	Black Bengal	0.011	-	1.000	0.993	0.999
	Jakharana	0.004	0.000	-	0.991	1.000
	Jamunapari	0.001	0.007	0.009	-	0.993
	Barbari	0.006	0.000	0.000	0.007	-
OPM-14	Sirohi	-	0.980	0.994	0.980	0.999
	Black Bengal	0.020	-	0.995	0.999	0.993
	Jakharana	0.006	0.005	-	0.995	1.000
	Jamunapari	0.020	0.001	0.005	-	0.995
	Barbari	0.010	0.007	0.000	0.005	-
OPA-01	Sirohi	-	0.990	0.999	0.977	1.000
	Black Bengal	0.010	-	0.999	0.998	0.999
	Jakharana	0.010	0.001	-	0.990	0.992
	Jamunapari	0.003	0.002	0.010	-	0.998
	Barbari	0.000	0.000	0.008	0.000	-
OPM-05	Sirohi	-	0.990	0.997	1.000	0.993
	Black Bengal	0.010	-	0.999	0.995	0.999
	Jakharana	0.003	0.001	-	0.990	0.999
	Jamunapari	0.000	0.005	0.010	-	0.996
	Barbari	0.007	0.010	0.010	0.004	-
OPP-08	Sirohi	-	0.999	0.999	0.990	0.977
	Black Bengal	0.001	-	0.990	0.999	1.000
	Jakharana	0.001	0.010	-	0.999	0.999
	Jamunapari	0.020	0.003	0.010	-	1.000
	Barbari	0.000	0.000	0.008	0.001	-
OPP-02	Sirohi	-	0.890	0.899	0.977	1.000
	Black Bengal	0.011	-	0.890	0.977	1.000
	Jakharana	0.100	0.110	-	0.999	0.992
	Jamunapari	0.020	0.003	0.010	-	0.999
	Barbari	0.000	0.000	0.008	0.001	-
Pooled over primers	Sirohi	-	0.987	0.997	0.985	0.987
	Black Bengal	0.013	-	0.997	0.997	0.999
	Jakharana	0.003	0.003	-	0.994	0.996
	Jamunapari	0.015	0.003	0.006	-	0.997
	Barbari	0.013	0.001	0.004	0.003	-

was observed between Black Bengal and Jakhrana (0.020), while Jamunapari and Jakhrana showed maximum genetic distance of 0.183.

Between-breed genetic distance based on band frequency (BF) estimates are given in Tables 5, 6. However, when pooled over primers, the minimum Between-breed genetic distance was observed between Black Bengal and Barbari (0.001), while Sirohi and Jamunapari showed maximum genetic distance of 0.015.

Results indicated that there were substantial genetic similarities, polymorphism and genetic distances within and between different goat breeds. Genetic similarity between different goat breeds may be utilized through a common breed improvement program. The genetic distances revealed that the phenotypic differences and geographical distances among the breeds are in conformity.

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