ISSN (Print) : 0974-6846
Indian Journal of Science and Technology, *Vol 8(28), DOI: 10.17485/ijst/2015/v8i28/56529, October 2015* ISSN (Online) : 0974-5645

Breed Characterization for Identification and Conservation of Black Bengal Goats of Jharkhand

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Abstract

Objective: Summary of breed characteristics of Black Bengal goats of Jharkhand (called as Jharkhand Black for ease) based on physical, cytogenetic and molecular parameters have been presented in the current study in order to characterize and conserve this unique germplasm. **Findings:** The physical parameters helped reveal the unique physical characteristics of Jharkhand Black goats with body weight of 18.64 and 17.75 kg respectively for the males and females. Cytogenetic characterization gave the length of all the autosomes (58) and sex (2) chromosomes of the total 60 chromosomes along with the karyotyping pattern of all 60 chromosomes. The X chromosome was found to be acrocentric and longest while the Y-chromosome was found to be smallest, dot like structure and suspected to be sub-metacentric. Molecular characterization showed the Mean na (observed number of alleles) was 2 while mean ne (effective number of alleles) was found to be 1.6935. Polymorphism information content (PIC) scores varied from 0.219 to 0.486 with over all over all mean 0.413. Evan's Watterson's Test of Neutrality indicated that F value for not even a single locus deviated from the standardized normal range of U95 and L95. **Conclusions:** The study might be further continued to provide additional information and evidence regarding the same which could be beneficial for conservation of this extremely important germplasm of Jharkhand and for the MAS.

Keywords: Acrocentric, Evan's Watterson Test of Neutrality, Germplasm, Karyotype, RAPD, Shannon's Index

1. Introduction

In India the goat population is 125.46 million which 23.02% of Asia's population. Goats have evolved and adapted to all diverse environment and carry unique combination of genes that define productive environment and adaptive capabilities. Phenotypic selection has created wide diversity of breeds of goat that adapted to different climatic condition and purposes. To overcome biological constraints such as sex limited gene expression, age limited traits and traits expensive to measure and genetic markers can potentially ameliorate the problem as they are independent of all the factors. The beauty of using genetic marker in breeding programme is that they mark chromosomal regions so can follow the inheritance of these regions from parent to offspring. Molecular tools are used to identify the desired allelic variants either through direct DNA test for desired variants or by DNA test for linked genetic marker associated with desired allele. Once animal have been classified based on desired alleles the information can be used in addition to phenotypic information to decide which animal to be used for breeding. This research was carried out on characterization of Black Bengal goat using cytogenetics and molecular study. The cytogenetic study involving karyotyping focused on chromosomal abnormalities

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in relation to abnormal phenotype and reproductive disorder. For molecular aspect, RAPD was used which is a PCR based important technique for identifying genetic variation involving the use of single arbitrary primer in a PCR reaction, resulting in amplification of many discrete DNA.

In Jharkhand Black Bengal goat have become choice of farmer and attributed mainly to low inputs, less risk, early return, faster multiplication, and easiness in management, maintenance on wide varieties on feeding resources and round the year market. Both the goat population's viz. Black Bengal and Black Bengal type goats of Jharkhand share much phenotypic similarities e.g. being black in color but differ in many morphological aspects such as body weight. Even the molecular study based on Microsatellite marker by Kumar. S[1] had also reported that the two goat populations showed significant divergence to each other in molecular study.

The present study undertakes to characterize the Black Bengal goat of Jharkhand (*called as Jharkhand black in the current article*) based on physical parameters, cytogenetic parameters and molecular parameters.

2. Materials and Methods

2.1 Physical Characterization

The physical parameters were taken as recorded by Sinha A. K[2].

2.2 Cytogenetic Characterization

The present study was undertaken on Black Bengal goats of Jharkhand (*Jharkhand Black*) maintained in Instructional Farm Small Ruminants, Ranchi Veterinary College, Birsa agricultural University, Ranchi. Out of the total animals present in this farm, 20 goats (10 Males and 10 females) were taken randomly from the breeding population for cytogenetic study. The short-term lymphocyte culture method reported earlier[3] was followed with slight modifications. Harvesting of the culture was followed by preparation of the slide and then by giemsa staining. A fixed number of parameters like, length, relative length, centromeric index, arm ratio and morphological index were measured. Finally the relative lengths of chromosomes were measured as percentage of the total genome length (excluding Y-chromosome). Since the data was not following normal distribution, the data were transformed to arc sin values and then subjected to standard statistical analysis[4].

2.3 Molecular Characterization

Blood samples of 50 unrelated individuals (10 ml each) from Jharkhand Black goat were collected from the respective places of their geographical distribution. Isolation and storage of DNA was followed by quality and quantity checks of DNA. PCR for standardization of PCR Mix and temperature optimization was done. It was followed by PCR for DNA amplification with the

Table 1. Breed characteristics of Jharkhand Black

Attributes	#Jharkhand Black	
Traits (Adult)	**Males**	**Females**
Body weight (kg)	18.64	17.75
Body length (cm)	50.98	47.87
Chest girth (cm)	59.53	55.30
Height at withers (cm)	55.24	53.47
Coat color	Black, white, grey	
Nose line	Slightly depressed	
Ears	Short and flat	
Ear length (cm)	10.43 ± 0.15	
Horns	Medium, directed upwards some times backwards	
Horn length (cm)	6.88 ± 0.131	
Age at first kidding	12.07 ± 0.59 month	
Kidding interval	8 ± 0.03 month	
Liter size (%) Single (S) Twins (T) Triplet (Tr), Quadruplet (Q)	Majority twins	
Milk yield (daily)	18.62 ± 1.47 kg (Lactation yield)	
Lactation length	78.44 ± 0.47 days	

#Sinha A. K.[2]

primers. For RAPD, total of 10 random primers were taken named as Sigma-1 to sigma - 10. Then Agarose Gel electrophoresis of PCR amplified DNA was done and RAPD bands were observed under UV Light. At last statistical Analysis of the Data was performed with Popgene and Arlequin software.

3. Results and Discussion

3.1 Physical Characterization

Black Bengal goat is small native breed of goat found in North east India and some part of Bangladesh. The goat is predominantly black in colour but sometime it may be brown or grey (30%) and white (10%). The goat hair is short, soft and skin is glossy and lustrous. The body is dwarf in size, straight back beard in both sexes. The body length, height and heart girth 54, 50 and 59 cm in bucks and 55, 49 and 59 cm in does respectively with well set and nicely developed udder. Ear is short and nearly upright. It is most prolific Indian breed which can be guessed from the fact that in this breed, multiple births is common with two, three or four kids born at a time with kidding twice in a year. Average litter size 2.1 average age at first kidding is 9-10 months. Its fine skin has high demand for good quality and shoe making.

3.2 Cytogenetic Characterization

The karyotype analysis of the Jharkhand black goat (Figure-1, Table 1) revealed, as expected, a diploid (2n) chromosome number of 60 in all complete metaphases examined. All the 29 autosomes were acrocentric and the RL of individual chromosomes varied from 5.19 to 5.25 of chromome 1 in male and female to 1.79 to1.78 of chromosome 29 in male and female, whereas the RL of the X and Y chromosomes were 5.95 and 1.47 respectively (Table 1). These values are in agreement with those previously reported for the species, thus confirming that the investigated animals did not differ from the standard of the species. The X chromosome was found to be acrocentric and longest which is in agreement with the earlier observations[5]. The Y-chromosome was found to be smallest, dot like structure and suspected to be sub-metacentric in the present investigation which is similar to the findings of Bhatia and Shankar[6] in Marwari goat and Ford et al.[7] and Berardino et al.[8], while Bunch et al.[9] reported that the Y chromosome may be metacentric or submetacentric but not acrocentric. The relative length of X chromosome was found to be 5.95±0.05, 5.57±0.05 and 5.76±0.05 % in male, female and pooled over sex respectively, and the relative length of Y chromosome was 1.47±0.03% which was in agreement with the findings of Pattnanyak and Patre[10].

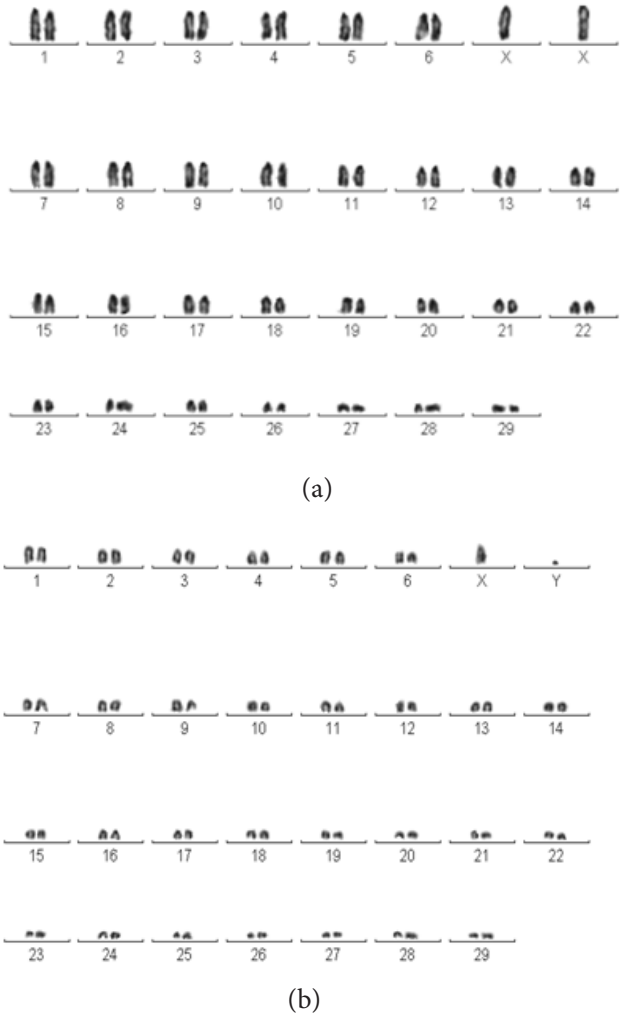

Figure 1. Mitotic-metaphase spread of Jharkhand male with its karyotype. (a) Black Bengal female. (b) Black Bengal male.

3.3 Molecular Characterization

A total of ten primers were taken out of which all others gave result except for primers 1 and 2 (Figure 2). Frequency ranged from 0.146 to 0.615 for allele 0 and from 0.385 to 0.854 for allele 1 (Table 2). It was observed that primers 3, 4 and 5 produced only one band while primers 6, 7, 8, 9 and 10 produced 4, 3, 3, 3 and 2 bands respectively. Similar findings were reported by Rehman et al.[11] during molecular characterization of Black Bengal and Jamunapari goat breeds by RAPD markers.

The highest level of gene frequency value (0.9286) was observed in allele 0 with primer BM1818 and the lowest frequency value (0.0714) was obtained in allele 1 using same primer.

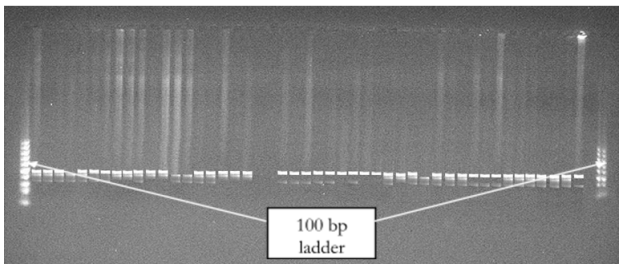

Figure 2. Jharkhand Black goat genomic (48samples) amplification with primer sigma 08.

Table 2. Mean ± SE of the relative length (%) of the chromosomes in male and female Black Bengal goat

Chr. Pair number	Mean ± SE	Mean ± SE
1	5.19±0.03$^{z''}$	5.25±0.04^{z}
2	5.00±0.02$^{z'}$	4.96±0.03^{y}
3	4.78±0.03^{z}	4.70±0.03^{w}
4	4.52±0.02^{y}	4.55±0.03^{x}
5	4.28±0.02^{x}	4.44±0.03^{v}
6	4.13±0.01^{w}	4.26±0.03^{u}
7	4.02±0.01^{v}	4.05±0.03^{t}
8	3.87±0.01^{u}	3.95±0.01^{s}
9	3.74±0.01^{t}	3.79±0.01^{r}
10	3.62±0.01^{s}	3.74±0.02^{r}
11	3.52±0.01^{r}	3.60±0.01^{q}
12	3.37±0.01^{q}	3.44±0.01^{p}
13	3.31±0.01^{p}	3.35±0.01^{o}
14	3.24±0.01^{o}	3.23±0.01^{n}
15	3.09±0.01^{n}	3.16±0.01^{m}
16	2.99±0.01^{m}	3.06±0.01^{l}
17	2.93±0.01^{l}	2.94±0.02^{k}
18	2.87±0.01^{k}	2.87±0.01^{j}
19	2.81±0.01^{j}	2.75±0.01^{i}
20	2.65±0.02^{i}	2.63±0.01^{h}
21	2.57±0.01^{h}	2.59±0.01^{h}
22	2.51±0.01^{h}	2.47±0.02^{g}
23	2.42±0.02^{g}	2.36±0.02^{f}
24	2.32±0.02^{f}	2.32±0.02^{f}
25	2.26±0.02^{f}	2.26±0.02^{e}
26	2.15±0.02^{e}	2.14±0.03^{d}
27	2.09±0.02^{d}	1.94±0.03^{c}
28	1.98±0.02^{c}	1.86±0.03^{b}
29	1.79±0.02^{b}	1.78±0.03^{a}
X	5.95±0.05$^{z'''}$	5.57±0.05$^{z'}$
Y	1.47±0.03^{a}	

**P£0.01, Value having same superscript in column did not differ significantly. N = 100

Polymorphism Information Content (PIC) or expected heterozygosity scores (Table 2.) varied from 0.219 to 0.486 with over all over all mean 0.413 in Jharkhand Black goat. Conservationists assume that genetic diversity in a population could adversely affect its short term viability[12]. According to Carvalho et al.[13] genetic diversity expressed inside and among population, can enhance adaptation to a particular habitat, expand the boundary of colonization, distribution and enhance species to survive in a wide variety of conditions. The heterozygosity lost might be achieved by outcrossing the population.

Mean n_a (observed number of alleles) was 2 in case Jharkhand Black while mean n_e (effective number of alleles) was found to be 1.6935 with maximum value of 1.9862 for locus SIGMA 06-1 and locus sigma 10-2, the minimum value of 1.3318 was observed for locus Sigma 08-1 (Table 2). These findings revealed that there was high level of allele diversity. These results did not agree with the findings of Kumar S[1] who observed lower level of allelic diversity in Jharkhand

Black goat indicating recent evolutionary history in a study of four goat populations with 25 microsatellite markers. The result of the present study with respect to level of allele's diversity for the evolutionary history in both the goat populations, agreed with the observation of Mac Hugh et al.[14] in European cattle.

Shannon's Information index[15], which measure the level of diversity, was moderately high with a mean of 0.5898. Mean Nei's Gene diversity value (h) i.e. overall expected heterozygosity[16] was 0.4022 for Jharkhand Black (Table 2). It was further found from Table 2 that the maximum and minimum Nei's gene diversity (h) were 0.4965 for loci sigma 06-1 and 10-2 and 0.249 for locus sigma 08-1 respectively.

In case of Evan's Watterson's Test of Neutrality (Table 2.), it was found that not even a single locus showed the F value beyond the standardized range of U95 and L95. Keliang et al.[17] observed that RAPD marker OPAI was found highly associated with many reproductive performances in Rex Rabbit.

According to Hepsibha et al.[18] RAPD technique has several advantages such as speed, low cost and the usage of small amounts of plant materials[19–21].

Similarly the results of Makari et al.[22] established that when an appropriately chosen set of primers is employed, RAPD analysis provides an alternative rapid, reproducible, and powerful genomic typing method for finding the genetic diversity in some chilli plant varieties of same species.

Table 3. Different parameters under RAPD for Jharkhand Black

Allele \ Locus	FREQUENCY		P.I.C.	GENETIC VARIATION					EVAN'S WATTERSON'S TEST OF NEUTRALITY			
	Allele 0	Allele 1			Na	ne	(h)	I	Obs. F	SE	L95	U95
SIG.03	0.323	0.677	0.413	48	2.000	1.704	0.413	0.604	0.587	0.027	0.501	0.959
SIG.04	0.344	0.656	0.395	48	2.000	1.653	0.395	0.584	0.605	0.025	0.501	0.959
SIG.05	0.250	0.750	0.395	48	2.000	1.600	0.375	0.562	0.625	0.027	0.501	0.959
SIG.06-1	0.594	**0.406**	0.496	48	2.000	1.986	**0.497**	0.690	0.504	0.026	0.501	0.959
SIG.06-2	0.271	0.729	0.375	48	2.000	1.600	0.375	0.562	0.625	0.027	0.504	0.959
SIG.06-3	**0.146**	**0.854**	**0.278**	48	2.000	1.385	0.278	**0.451**	0.722	0.026	0.501	0.959
SIG.06-4	0.396	0.604	0.469	48	2.000	1.917	0.478	0.671	0.522	0.026	0.501	0.959
SIG.07-1	0.250	0.750	0.353	48	2.0000	1.546	0.353	0.538	0.647	0.027	0.504	0.959
SIG.07-2	0.344	0.656	0.429	48	2.000	1.753	0.430	0.621	0.570	0.026	0.504	0.959
SIG.07-3	0.302	0.698	0.478	48	2.000	1.704	0.413	0.604	0.587	0.026	0.501	0.959
SIG.08-1	0.198	0.802	0.249	48	2.000	1.332	**0.249**	0.415	0.751	0.026	0.501	0.959
SIG.08-2	0.240	0.760	0.469	48	2.000	1.546	0.353	0.539	0.647	0.027	0.501	0.959
SIG.08-3	0.271	0.729	0.413	48	2.000	1.704	0.413	0.604	0.587	0.027	0.501	0.959
SIG.09-1	0.302	0.698	0.375	48	2.000	1.600	0.375	0.562	0.625	0.026	0.501	0.959
SIG.09-2	0.240	0.760	0.395	48	2.000	1.653	0.395	0.584	0.605	0.027	0.501	0.959
SIG.09-3	0.292	0.708	0.457	48	2.000	1.843	0.458	0.650	0.543	0.026	0.501	0.959
SIG.10-1	0.521	0.479	0.491	48	2.0000	1.9692	0.4922	0.6853	0.5078	0.0252	0.5035	0.9592
SIG.10-2	**0.615**	**0.385**	**0.496**	48	2.000	1.986	**0.497**	**0.690**	0.504	0.027	0.501	0.959

SIG.- Sigma, P.I.C.- Polymorphic Information Content, Na- Observed number of alleles, h- Nei's gene diversity Ne- Expected number of alleles, I- shannon's index

4. Conclusion

It is a famous breed known for high prolificacy, superior chevon quality, best quality Skin, early sexual maturity, low kidding interval and very good adaptability. Multiple births are common for this breed of goat like twin or triplets. Phenotypic characterization shows distinct dissimilarities between the breeds. Karyotypes of the animals investigated resulted chromosomally normal and thus the result is useful and conclusive. Since the consequences of chromosomal polymorphisms which was detected in the current study specially for the sex chromosome, are detrimental for the reproductive and productive efficiency of the breed, characterization of genetic resources of a given region is essential to avoid possible intermating with other breeds or genetic types that could lead to a reduction in fertility or to a loss of the ancient genetic combination. Molecular characterization was mainly done on breeds raised in marginal agricultural areas in order to assist in situ conservation and was important with respect to puzzle of goat genetic diversity and conservation and also for marker assisted selection. All measures of genetic variations i.e. observed number of alleles, Shannon's Information indicated high polymorphism across the loci, suggesting suitability of these markers for genetic diversity studies in goat. Further in the current scenario of utility based breeding systems, the conservation of native germplasm is also important for ecological stability. Current findings of phenotypic, cytogenetic and molecular characterization coupled with more research works could be extremely important for characterization, conservation and selection.

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