Karyological Studies on Tho-tho (*Bos indicus*): an Indigenous Cattle of Nagaland

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Abstract

Tho-tho cattle is short to medium stature black color animal available in different hilly pockets of Nagaland. Body is mostly covered with short and glossy hairs. Coat color is either pure black or skew bald, neck is long and fine, horns are curved with tips pointed upward. Udder is small and hump is prominent in this animal. Tho-tho cattle play an important role in the socio-cultural life of the local tribal people and are considered as the ceremonial animal of various social rituals. The diploid chromosome number in Tho-tho cattle was observed to be 60 in all complete metaphase spreads. The normal karyotype of Tho-tho cattle showed that all the autosomes are acrocentric whereas among the sex chromosome X was found to be sub-metacentric and Y as smallest acrocentric. The relative length of the autosomal chromosomes varied from 5.24% of chromosome number 1 to 1.88% of chromosome number 29 and the relative length of the X and Y chromosomes were 4.91 and 1.79%, respectively. The average centromeric index in Tho-tho cattle was about 36.18% and the arm ratio was 1.78.

1. Introduction

Situated in the north-eastern hilly region of India Nagaland is considered as a biodiversity hot spot. In the hilly forest of Nagaland different varieties of locally available jungle forages offer the opportunity to rear livestock under free range system with minimum inputs. Livestock rearing is an important component of Naga livelihood. Tho-tho cattle is short to medium stature black color animal (Figure 1) available in different hilly pockets of Nagaland, especially in Kohima and Phek districts.

Body is mostly covered with short and glossy hairs. Coat color is either pure black or black with white spot; long and fine neck, horns are blackish and curved upward with pointed tips. Udder is small and hump is prominent in this animal (Dhali et al., 2006). Though Tho-tho is the popular name, this animal is also known by different other names with location specific tribal names. These cattle are primarily used for meat and dung purposes. However, the evidences are there that these cattle are also used as milk and draught purposes occasionally. Thus they play an important role in socio-cultural life of the local tribal people and are considered as the ceremonial animal of various social rituals. The total population of Tho-Tho cattle is estimated about 89,337 and is recognized as descript indigenous cattle breed of India (Singh, 2006). The period of domestication of these cattle breed is not clear. However, the local people have been rearing this animal since last 500 years. Tho-tho cattle is having significant distinct phenotype as compared to the other breeds of cattle due to geographical boundaries and environmental uniqueness. Since the traits are heritable in nature it needs to be evaluated cytogenetically as well as at molecular level. Therefore, the present study was focused on the chromosome morphology and cytogenetic analysis in this breed. The information generated will be helpful for providing the basis for comparative analysis with the other breeds of *Bos indicus*. Since, there is no report of chromosomal study on this breed of cattle till date; this cytogenetic study will be useful to generate a base for further detail analysis using molecular technique and also in selecting the animals with chromosomal abnormalities, if any, which might affect the productive and reproductive performance and overall herd economic loss. Ultimately, this will be useful for genetic conservation and propagation of Tho-tho cattle.

2. Materials and Methods

The blood sample (3 ml) was collected under aseptic condition in vacutainer tubes from jugular vein of Tho-tho cattle

Figure 1: Tho-tho cattle

from various places of Kohima district and from institute farm of National Research Center on Mithun, Jharnapani. Blood lymphocyte culture technique was applied following Yadav and Balakrishnan (1985) with slight modification. A volume of 0.5 ml blood was cultured in each tube containing 6 ml Ham's F-10 (cat. no. 12-618F) cell culture media, which was supplemented with 15% fetal calf serum (Sera laboratories, cat. no. EU-000-HI) and pokeweed mitogen (L8777-10MG) at 2.5 µg ml^{-1} added with antibiotics penicillin (cat. no. P-8431) and streptomycin (cat. no. S-9137), and incubated for 72 h at 37°C. The culture tubes were shaken at 5-6 h interval for the uniform distribution of cell in the media. Two drops of colchicine (100 µg ml^{-1}) was added 45 minutes prior to the harvesting of the culture. Harvesting was done by transferring the media into the centrifuge tube and was spun at 1,500 rpm for 10 minutes. The supernatant was removed and the cell pellet was treated with pre-warm hypotonic solution (0.075 M KCl) and incubated at 37°C in water bath for 25 minutes. Few drops of freshly prepared and chilled Carnoy's mixture, i.e. fixative (3:1, glacial acetic acid and methanol) were added to stop the action of hypotonic solution. The cell button was washed with fixative till the supernatant became clear in about 4-5 series of washing. The cell button was used for preparing the slide and air dried until it was stained. Slides were stained in 2% Giemsa for 20 minutes and screened under microscope (Nikon 90i) firstly at 10X followed by 100X (1,000 magnification). The good metaphase spreads from the slide were selected and photographs were taken. The measurements of chromosomes were done for identification and pairing of homologous chromosomes using ImagePro 9.1 software. According to the size, length and structure of chromosome the karyotype was constructed. Using these values the parameters for cytogenetic analysis such as relative length (length of the individual chromosome/total length of all chromosome x 100), centromeric index (Ci=100 x short arm/total length), arm ratio (r=Long arm/short arm) and also the size of chromosome and contribution to the total genome were calculated. These parameters were used not only for the construction of karyotype and ideogram, but also required for morphology description of the chromosome.

3. Results and Discussion

The chromosome number in Tho-tho cattle was observed to be 2n=60 in all complete metaphase examined in the present study. Figure 2 shows metaphase plate (a) and karyotype (b) of female Tho-tho cattle. Figure 3 shows metaphase plate (a) and karyotype (b) of male Tho-tho cattle.

This report on chromosome number of Tho-tho cattle is in agreement with the earlier report on normal chromosome number of various breeds of *Bos indicus* (Kosarcic et al., 2005; Balaji et al., 2006; Kumarasamy et al., 2008; Jantarat et al., 2009). The normal karyotype of Tho-tho cattle shows that all the autosomes are acrocentric chromosome and the sex chromosome, i.e. X chromosome was sub-metacentric chromosome and Y chromosome was the smallest acrocentric chromosome. The relative length of the autosomal chromosomes varied from 5.24 % of chromosome number 1 to 1.88% of chromosome number 29 and the relative length of X and Y chromosomes were 4.91 and 1.79%, respectively (Table 1).

The variation of relative length in autosome is similar to the Deoni cattle breed (Balaji et al., 2006). In Tho-tho cattle the

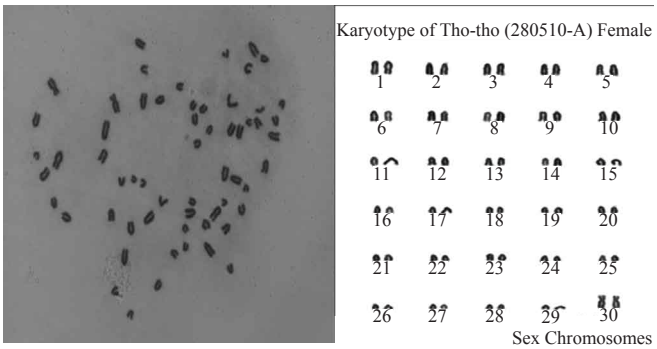

Figure 2: Metaphase plate (a) and karyotype (b) of female Tho-tho cattle

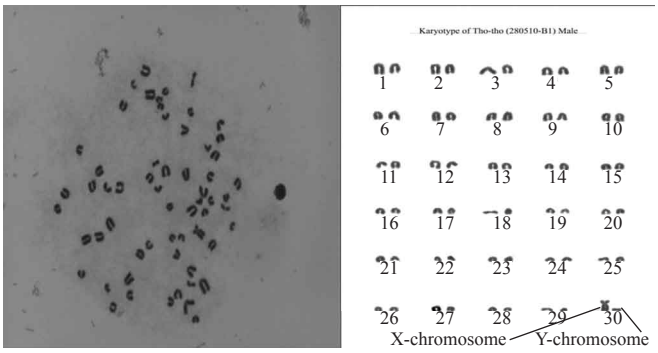

Figure 3: Metaphase plate (a) and karyotype (b) of male Tho-tho cattle

Table 1: Morphometric characteristics of Tho-tho cattle chromosomes

Chromosome number	Relative length (%)	Centromeric index (%)	Arm ratio
1	5.24		
2	4.66		
3	4.47		
4	4.31		
5	4.21		
6	4.10		
7	4.00		
8	3.88		
9	3.73		
10	3.66		
11	3.61		
12	3.47		
13	3.33		
14	3.21		
15	3.12		
16	3.02		
17	2.94		
18	2.87		
19	2.79		
20	2.67		
21	2.64		
22	2.54		
23	2.48		
24	2.35		
25	2.28		
26	2.24		
27	2.19		
28	2.08		
29	1.88		
X	4.91	36.18	1.78
Y	1.79		

largest chromosome in the genome is chromosome 1 which is similar to that of Thai's native cattle (Jantarat et al., 2009), unlike that of Deoni cattle breed and Umblachery cattle breed where X chromosome is the largest in the genome. The morphology of chromosome was ascertained by estimating the parameters like centromeric index and arm ratio on bi-armed X chromosomes. The average centromeric index in Tho-tho cattle was found to be 36.18%. According to Levan et al. (1964), value falls within the range of 25-37.5%, and according to (Gurrea, 1986), value falls within the range of 25.1-40%. Bi-armed X chromosome is categorized as sub-metacentric chromosome. Similarly the arm ratio was also calculated in the bi-armed X chromosome and the value was calculated to be 1.78 which lies in the range of 1.67-3 (Levan et al., 1964) and 1.50-2.99 (Gurrea, 1986), which add up to the confirmation of X chromosome being sub-metacentric as shown in Table 1. Based on the value of the cytogenetic analysis an ideogram was prepared (Figure 4).

This cytogenetic parameters of the present study for Tho-tho breed of cattle are in agreement with those previously reported for *Bos indicus*, thus confirming that karyotype profile of investigated animals do not differ from standard karyotype of bovine species. However, the present study on chromosomes of Tho-tho cattle will be a platform for further comparative studies with other breeds of same species by molecular cytogenetics and molecular techniques.

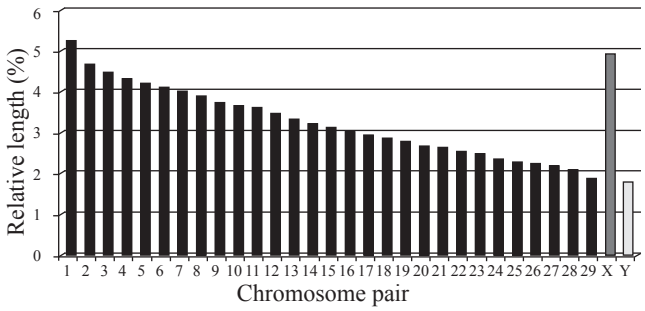

Figure 4: Ideogram representing the relative length of chromosomes of Tho-tho cattle

4. Conclusion

Tho-tho cattle, a newly characterized breed of the north-eastern region of India, have the diploid chromosome number of 60 in all complete metaphase spreads. The normal karyotype showed that all the autosomes are acrocentric whereas among the sex chromosome X was found to be sub-metacentric and Y as smallest acrocentric. The relative length of the autosomal chromosomes varied from 5.24% of chromosome number 1 to 1.88% of chromosome number 29 and the relative length of the X and Y chromosomes were 4.91 and 1.79%, respectively. The average centromeric index in Tho-tho cattle was about 36.18% and the arm ratio was 1.78.

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