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CYTOGENETIC STUDIES IN MALNAD GIDDA CATTLE

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Cytogenetic investigation was carried out in two breed groups of cattle, viz. Malnad Gidda (*B.indicus*) and Jersey (*B.taurus*), in an effort to compare the karyomorphological pattern between Malnad Gidda cattle and Jersey. Blood samples were collected from Malnad Gidda cattle maintained by farmers of Malnad region of Chikmagalur District of Karnataka and from Jersey cattle maintained at Livestock Breeding and Training Centre, Dharwad, Dept. of A.H & V.S, Govt. of Karnataka.

Metaphase spreads were obtained by short term whole blood lymphocyte culture using standard techniques. A normal chromosomal complement of 2n=60 (58 autosomes and 2 sex chromosomes) was observed in all Malnad Gidda and Jersey cattle. X chromosome was submetacentric in both breeds and Y chromosome was acrocentric in Malnad Gidda and metacentric in Jersey cattle. X chromosome was the longest chromosome, while first autosome was second longest and Y chromosome was shortest chromosome in both Malnad Gidda and Jersey cattle. The relative length (RL) of autosomes progressively decreased from 4.85 ± 0.05 to 1.85 ± 0.03 per cent and from 4.95 ± 0.05 to 1.58 ± 0.03 per cent in male and female Malnad Gidda cattle, respectively; and from 5.00 ± 0.07 to1.80 ± 0.04 per cent and from 4.76 ± 0.08 to1.83 ± 0.05per cent in male and female Jersey cattle, respectively. The X chromosome contributed 5.09 ± 0.05 and 5.15 ± 0.05 per cent in male and female Malnad Gidda, respectively; and 5.12 ± 0.05 per cent and 4.86 ± 0.09 per cent in male and female Jersey cattle, respectively, where as the Y chromosome contributed 1.70 ± 0.03 and 1.69 ± 0.05 per cent towards total genome in male Malnad Gidda and male Jersey cattle, respectively. The mean centromeric index (CI) of X chromosome was 0.32 ± 0.01 and 0.33 ± 0.01 in Malnad Gidda and Jersey cattle, respectively. The G-banding pattern of Malnad Gidda cattle was almost similar with minor differences to that of Amrithmahal, Hallikar and Deoni cattle, reported earlier.

Key words: Malnad Gidda, Karyotyping, Relative Length (RL), Centromeric Index (CI), Arm Ratio (AR)

Livestock rearing is an integral part of the Indian rural agriculture, over the past many centuries. Integrated crop-livestock farming has been an age-old practice in Karnataka. The farmers have excelled in livestock breeding, which is evident from the number of breeds of livestock developed in the state. At present in Karnataka, there are five well-defined breeds of cattle apart from non-descript local/native cattle distributed all over the state. One such breed of cattle is Malnad Gidda found in the Western Ghats of Karnataka (Fig. 1). Generally, 'Gidda' refers to dwarf, although the body is compact and well built proportionately to the height. Malnad Gidda cattle are confined to heavy rainfall and hilly areas of Chikmagalur, Dakshina Kannada, Hassan, Shimoga, Udupi and Uttara Kannada of coastal and transitional agro-climatic zones of Karnataka (Ashok, 2000). Malnad Gidda cattle are known for their grazing ability at higher altitude, high disease resistance and endurance to adverse climatic conditions such as heavy rainfall and hot humid condition. Adoption of crossbreeding on an extensive scale in these regions during the past several decades and frequent inter-

mating among cattle breeds located in neighbouring areas has resulted in significant decrease in the number of Malnad Gidda cattle across the Western Ghats of Karnataka. It is most likely that they may serve as valuable genetic resources for future exploitation of genes for high disease resistance through genetic engineering. Thus, efforts are on the anvil to conserve these animals in Karnataka. Keeping these facts in view, the present study was contemplated for cytogenetic characterization of Malnad Gidda and to compare it with chromosomal profile of Jersey cattle.

Figure 1: Malnad Gidda female and male cattle

MATERIALS AND METHODS

Sampling site and quantity of rice gruel

In the present study, Cytogenetic investigation was carried out on 20 Malnad Gidda cattle maintained by farmers of Malnad region of Chikmagalur District of Karnataka. Twelve Jersey cattle maintained at Livestock Breeding and Training Centre, Dharwad, Dept. of A.H & V.S, Govt. of Karnataka, were also used for comparative study. The cattle selected for the cytogenetic study comprised of 10 Malnad Gidda males, 10 Malnad Gidda females, 6 Jersey males and 6 Jersey females. A five ml sterile Sodium heparinised vacutainer tube was used to collect the blood from animals. Four and a half to five ml of blood was directly drawn into the vacutainer tube from the jugular vein. Short term lymphocyte culture was carried out to obtain metaphase chromosomal spreads using standard techniques (Eldridge, 1985) with some modifications. Colchicine was used at the rate of five µg per 10 ml of medium to harvest the lymphocytes in cell culture at metaphase stage. Ethidium bromide was added at the rate of 300 µl per 10 ml of medium as an inhibitor of chromosome contraction to get lengthier chromosomes (Hsu et al. 1973; Ikeuchi and Sasaki, 1979). Hypotonic treatment was done by using 0.075 M Potassium chloride (KCl) for a duration of 45 minutes to get swollen lymphocytes and thereby to obtain better chromosomal spreads. For karyotyping, the slides with chromosomal spreads were placed on hot plate (60^0 C) for drying and incubated at 65^0C overnight for G- banding. G- banding was carried out using 0.1 per cent trypsin working solution and staing was done using 10 per cent buffered Giemsa stain (pH- 6.8) for 3-5 minutes. Good metaphase spreads were photographed using a bright field microscope (Leica DMLS), attached with 35 mm camera (Leica Wetzlar, MPS 32). For photography Kodak Gold 200 color film was used. For both the cattle breed groups karyotyping was carried out and the following linear parameters of chromosomes were determined from their mean lengths by the quotients.

$$\text{Relative Length (RL)} = \frac{\text{Length of individual chromosome}}{\text{Total length of genome including sex chromosome}} \times 100$$

$$\text{Centromeric Index (CI)} = \frac{\text{Length of Short arm}}{\text{Total length of its chromosome}}$$

$$\text{Arm Ratio (AR)} = \frac{\text{Length of Long arm}}{\text{Length of Short arm}}$$

Based on the relative length of chromosomes the ideograms were also prepared for the both groups of cattle breeds. Appropriate statistical analyses were carried out as described by Snedecor and

Cochran (1967). The level of significance was fixed at 5 per cent ($p \leq 0.05$).

RESULTS AND DISCUSSION

Model chromosome number and morphology

The karyotypes from female and male Malnad Gidda cattle (Fig. 2) and female and male Jersey cattle (Fig. 3) possessed diploid chromosome number of 2n=60 (58 autosomes and 2 sex chromosomes), as established earlier (Raghunandanan and Mukundan, 1991; Shashikanth, 1992; Choudhury et al., 1997; Sarkhel and Katpatal, 1998; Dayananda, 2000; Appannavar et al., 2004; Sahoo et al., 2004). Karyotypes of Malnad Gidda cattle (Fig. 2) and Jersey cattle (Fig. 3) revealed that all the autosomes were acrocentric and X chromosomes were submetacentric in morphology. Similar findings were reported by Gupta et al. (1974), Desai et al. (1984), Raghunandanan and Mukundan, (1991), Shashikanth, (1992), Choudhury et al. (1997), Nagpure et al. (2001) and Sahoo et al. (2004). However, differences were observed in the morphology of Y chromosome between Malnad Gidda and Jersey cattle. The Y chromosome of Malnad Gidda (B. indicus) exhibited acrocentric morphology in contrast to the metacentric morphology of Y chromosome of Jersey (B. taurus) cattle, which was in accordance with the observations of Potter and Upton (1979), Desai et al. (1984), Raghunandanan and Mukundan (1991) and Choudhury et al. (1997). The difference in the structure of Y chromosome between zebu and taurus cattle would help in identification of male lines used in the crossbreeding programme.

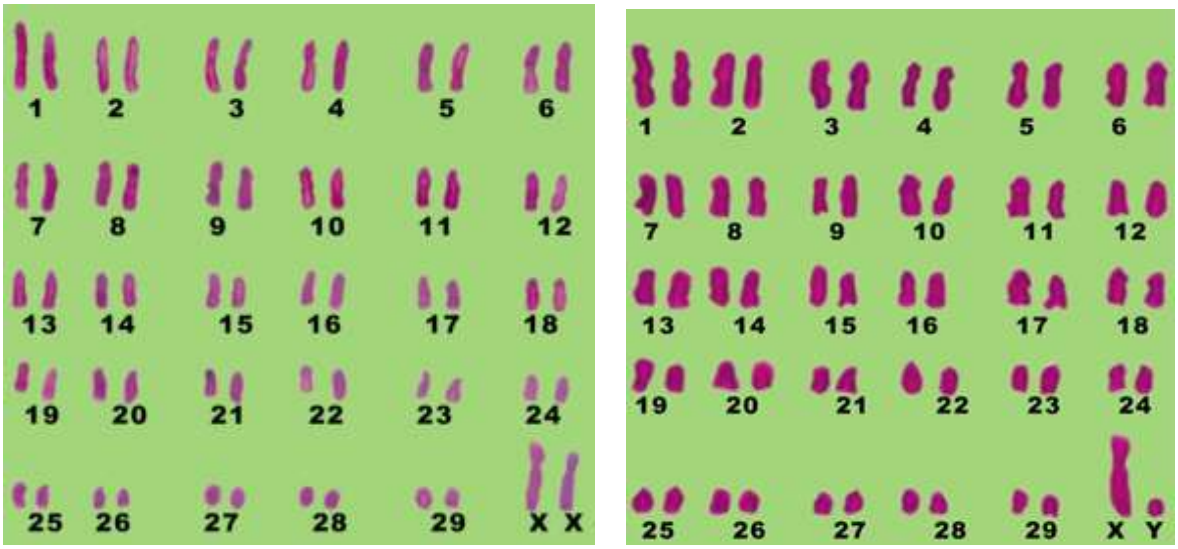

Figure 2 : Malnad Gidda Female and Male Karyotyping

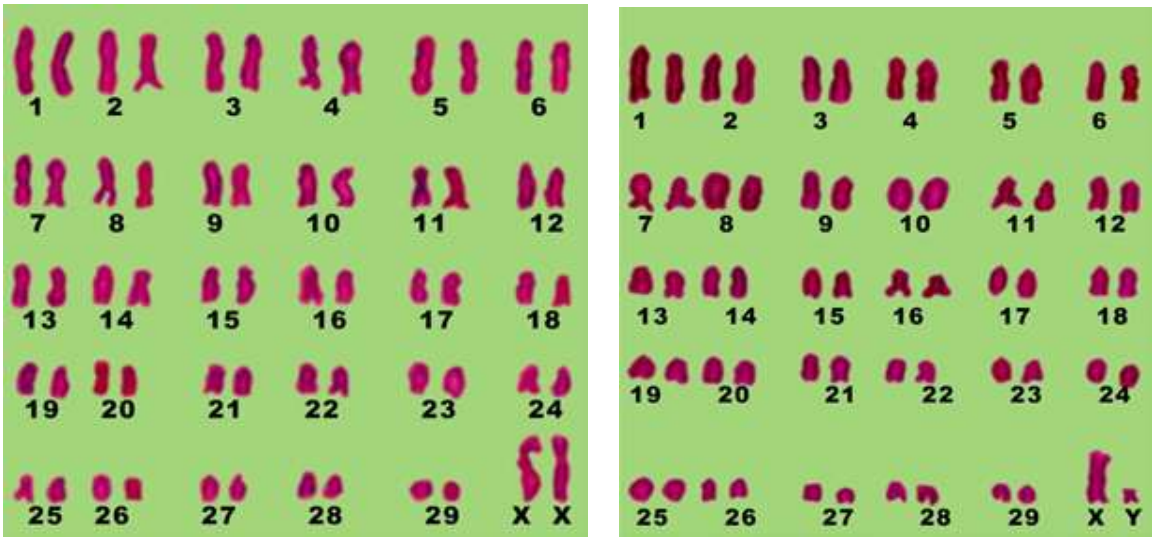

Figure 3: Jersey Female and Male Karyotyping

Relative length (RL)

The relative length of each chromosome pair was expressed as a percentage of haploid genome and based on RL, ideograms were constructed. The relative length of autosomes formed a continuous series ranging from 4.85 ± 0.05 to 1.85 ± 0.03 per cent and 4.95 ± 0.05 and 1.58 ± 0.03 per cent in male and female Malnad Gidda cattle, respectively. These values were apparently little different to those of other breeds of Indian cattle (Raghunandanan and Mukundan, 1991; Choudhury *et al*., 1997; Sarkhel and Katpatal, 1998; Nagpure *et al*., 2001; Appannavar *et al*., 2004; Sahoo *et al*., 2004). Similarly, the relative length of autosomes ranged from 5.00 ± 0.07 to 1.80 ± 0.04 per cent and 4.76 ± 0.08 to 1.83 ± 0.05 per cent in male and female Jersey cattle, respectively. These values were similar to the findings of other workers in Jersey cattle (Desai *et al*, 1984; Raghunandanan and Mukundan, 1991; Choudhury *et al*., 1997; Sarkhel and Katpatal, 1998). The relative lengths of the two X chromosomes of female Malnad Gidda were 5.16 ± 0.05 and 5.14 ± 0.05 per cent, respectively, whereas, it was 5.09 ± 0.05 per cent in male Malnad Gidda cattle. Similarly, the relative length of X chromosome was 4.87 ± 0.09 and 4.84 ± 0.09 per cent for the two X chromosomes in female Jersey cattle, whereas it was 5.12 ± 0.08 per cent in male Jersey cattle. The earlier findings reported by other workers in Jersey and other breeds of cattle ranged from 5.01 to 7.28 per cent (Desai *et al*, 1984; Raghunandanan and Mukundan, 1991; Choudhury *et al*., 1997; Nagpure *et al*., 2001; Appannavar *et al*., 2004; Sahoo *et al*., 2004). In both Malnad Gidda and Jersey cattle, the X chromosome was the largest chromosome among the whole chromosomal complement. This finding was contrary to the findings of other workers in few other zebu cattle breeds. Nagpure *et al*. (2001), Appannavar *et al*. (2004) and Sahoo *et al*. (2004) recorded the X chromosome as the second largest chromosome in Hariana, Deoni and Malvi breeds of cattle, respectively. However, Choudhury *et al*. (1997) in Assam local male cattle, Raghunandanan and Mukundan (1991) in Kerala local cattle and Sahai and Mathur (1981) in Sindhi and Hariana cattle reported findings similar to that observed in the present study. In the present study, the relative length of Y chromosome was 1.70 ± 0.03 and 1.69 ± 0.05 per cent in male Malnad Gidda and male Jersey cattle, respectively. These findings were in agreement with the earlier report by Nagpure *et al*. (2001) in Hariana cattle. However, these findings were apparently little different from the findings by other workers in different breeds of cattle (Raghunandanan and Mukundan, 1991; Choudhury *et al*., 1997; Appannavar *et al*., 2004; Sahoo *et al*., 2004). In male Malnad Gidda (*B. indicus*) cattle, Y chromosome was the smallest acrocentric chromosome. This observation was in agreement with the findings of Sarkhel (1989), Vij *et al*. (1997), Appannavar *et al*. (2004) and Sahoo *et al*. (2004) in Gir, Nagori, Deoni and Malvi breeds of cattle, respectively. However, Raghunandanan and Mukundan (1991) reported Y chromosome position between 15^{th} and 16^{th} autosomes in local cattle of Kerala, where as in Assam local cattle and Hariana cattle it was reported to be similar to 26^{th} and 28^{th} pair of autosomes, respectively, by Choudhury *et al*. (1997) and Nagpure *et al*. (2001), while Vijh *et al*. (1996) suggested that the Y chromosome could be placed between 27^{th} and 28^{th} autosome pairs in Rathi cattle. The slight to moderate differences noticed between the reports of the earlier workers and the current study may indicate breed differences at cytogenetic level. Similarly in male Jersey cattle also, Y chromosome was recorded as the smallest chromosome with metacentric morphology. Desai *et al*. (1984), Raghunandanan and Mukundan (1991) and Choudhury *et al*. (1997) also reported metacentric morphology of Y chromosome in male Jersey cattle. However, Desai *et al*. (1984) and Raghunandanan and Mukundan (1991) observed the position of the Y chromosome to be between 28^{th} and 29^{th} and 15^{th} and 16^{th} pair of autosomes, respectively, while Choudhury *et al*. (1997) reported the position of the Y chromosome to be the 26^{th} pair of autosomes, in zebu cattle breeds. In the present study, the mean relative length of

chromosomes for both Malnad Gidda and Jersey cattle was compared using Student's 't'-test and no significant difference was found. In general no apparent differences in chromosomal complement and relative length of individual chromosomes were observed between Malnad Gidda and Jersey cattle. These results were in agreement with the findings by Desai *et al*. (1984), Raghunandanan and Mukundan (1991), Choudhury *et al*. (1997) and Sarkhel and Katpatal (1998).

Centromeric Index (CI)

In this study, the mean centromeric index for the X chromosome was 0.32 ± 0.01 and 0.33 ± 0.01 in Malnad Gidda and Jersey cattle, respectively, and that of Y chromosome of male Jersey cattle was 0.41 ± 0.01. This indicated the submetacentric morphology of X chromosome, as the centromere was a little away from the mid-point in both the cattle breeds under study. However, the Y chromosome of male Jersey cattle was categorized as metacentric due to the mid-point positioning of centromere. These values are in agreement with the findings of Potter *et al.* (1979), Slawomirski *et al*. (1979), Desai *et al*. (1984), Raghunandanan and Mukundan (1991) and Sahoo *et al*. (2004).

Arm Ratio (AR)

In the present study, the mean arm ratio for the X chromosome was 2.12 ± 0.07 and 2.21 ± 0.12 in Malnad Gidda and Jersey cattle, respectively. The Y chromosome of male Jersey cattle had the mean arm ratio of 1.54 ± 0.09. The reported figures for X and Y chromosomes by earlier workers ranged from 1.69 to 2.08 and 1.21, respectively (Raghunandanan and Mukundan, 1991; Sahoo *et al*., 2004). However, all these findings confirm the submetacentric morphology of X chromosome in both Malnad Gidda and Jersey cattle and metacentric morphology of Y chromosome in male Jersey cattle.

G-banding pattern of Malnad Gidda cattle

The G-banding pattern of Malnad Gidda cattle (Fig. 4) in the present study was almost similar to that of Amrith Mahal and Hallikar (Shashikanth, 1992) and Deoni (Appannavar *et al.*, 2004) cattle. However, minor differences were observed in the banding pattern of chromosome numbers 1, 4, 7, 9, 11, 16, 17, 26, 27 and 29 in these breeds. The chromosome No. 1 was the second largest chromosome next to X chromosome with four to five dark bands in Malnad Gidda cattle, whereas it was longest chromosome with one dark band in Amrith Mahal, Hallikar (Shashikanth, 1992) and Deoni cattle (Appannavar *et al*., 2004). The chromosome No. 4 had three proximal and one distal dark band in Malnad Gidda cattle, whereas Amrith Mahal and Hallikar (Shashikanth, 1992) and Deoni cattle (Appannavar *et al*., 2004) were reported to have one proximal and two distal dark bands. The chromosome No. 9 showed distinct difference where four dark bands were distributed throughout the chromosome in Malnad Gidda, whereas it was darkly stained throughout in Amrith Mahal, Hallikar (Shashikanth, 1992) and Deoni cattle (Appannavar et *al*., 2004).

The chromosome No. 11 had five dark bands in Malnad Gidda cattle, whereas Deoni cattle had only three dark bands (Appannavar *et al*., 2004). The chromosome Nos. 16 and 17 had three dark bands distributed throughout the chromosome in Malnad Gidda cattle, whereas Shashikanth (1992) observed one to two dark bands only in Amrith Mahal and Hallikar. The chromosome Nos. 26 had one dark band in Malnad Gidda cattle, whereas Deoni (Appannavar *et al*., 2004), Amrith Mahal and Hallikar cattle (Shashikanth, 1992) had two dark bands. The chromosome Nos. 27 and 29 had two dark bands in Malnad Gidda cattle, whereas only one dark band was reported in Deoni cattle (Appannavar *et al*., 2004). The smaller autosomes of Malnad Gidda (*Bos indicus*) stained slightly darker than rest of the autosomes similar to the observation made by Halnan *et al*. (1981), Shashikanth (1992) and Appannavar *et al*. (2004). The X chromosome of Malnad Gidda exhibited a distinct pale central region of q arm, an observation in contrast to the report of Halnan *et al*. (1981), but similar to that of Shashikanth (1992) and Appannavar *et al*. (2004). The majority of autosomes of

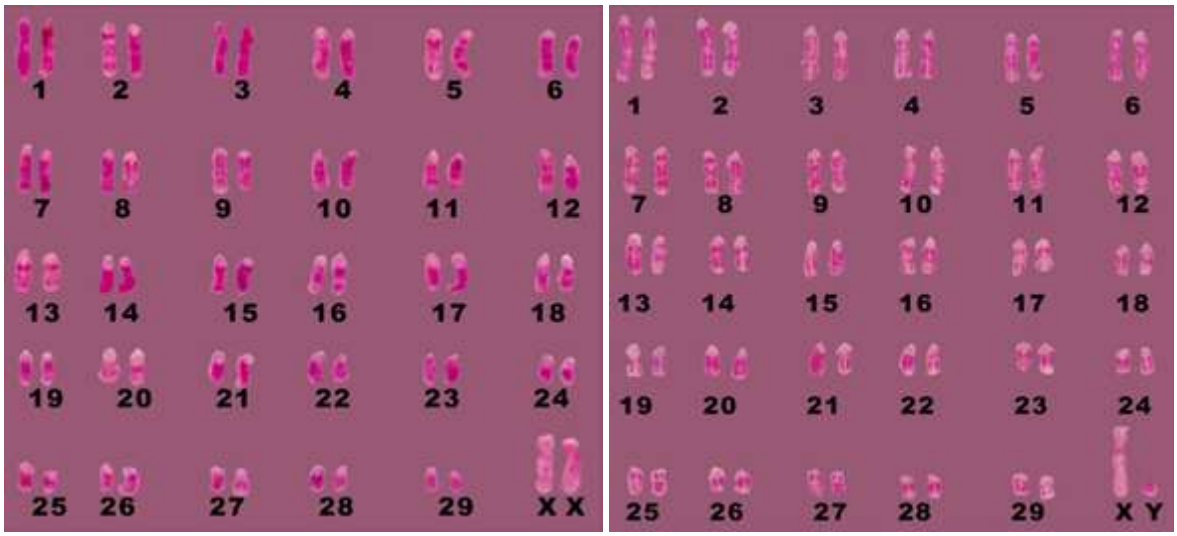

Figure 4: Malnad Gidda G-Banding pattern

Malnad Gidda cattle did not exhibit positive G-bands at their centromeric region, while X chromosome showed a faint staining of centromeric region with G-banding technique. These results are similar to the findings of Shashikanth (1992) and Appannavar *et al*. (2004). The Y chromosome was the smallest acrocentric chromosome with dark centromere and stained dark throughout, which was in agreement with the observation made by Appannavar *et al*. (2004). The above contrasting findings by different workers on G-banding pattern of zebu cattle need further investigation. Since these zebu breeds are related to each other such a major variation in the chromosome banding pattern is difficult to explain. One of the possible causes could be the variation in the technique followed by the different workers. Simultaneous cytogenetic evaluation under identical laboratory conditions and techniques would probably bring out the karyological differences, if any.

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