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ORIGINAL PAPER

Assessment of adaptability of zebu cattle (*Bos indicus*) breeds in two different climatic conditions: using cytogenetic techniques on genome integrity

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Abstract The aim of this study was to evaluate the genome integrity so as to assess the adaptability of three breeds of indigenous cattle reared under arid and semi-arid regions of Rajasthan (Bikaner) and Haryana (Karnal) India. The cattle were of homogenous group (same age and sex) of indigenous breeds viz. Sahiwal, Tharparkar and Kankrej. A total of 100 animals were selected for this study from both climatic conditions. The sister chromatid exchanges (SCE's), chromosomal gaps and chromatid breaks were observed in metaphase plates of chromosome preparations obtained from in vitro culture of peripheral blood lymphocytes. The mean number of breaks and gaps in Sahiwal and Tharparkar of semi-arid zone were 8.56 ± 3.16, 6.4 ± 3.39 and 8.72 ± 2.04, 3.52 ± 6.29, respectively. Similarly, the mean number of breaks and gaps in Tharparkar and Kankrej cattle of arid zone were 5.26 ± 1.76, 2.74 ± 1.76 and 5.24 ± 1.84, 2.5 ± 1.26, respectively. The frequency of SCEs in chromosomes was found significantly higher ($P < 0.05$) in Tharparkar of semi-arid region (4.72 ± 1.55) compared to arid region (2.83 ± 1.01). Similarly, the frequency of SCEs was found to be 4.0 ± 1.41 in the Sahiwal of semi-arid region and 2.69 ± 1.12 in Kankrej of arid zone. Statistical analysis revealed significant differences ($P < 0.05$) amongst the different zones, i.e. arid and semi-arid, whereas no significant difference ($P > 0.05$) was observed in the same zone. The analysis of frequency of CAs and SCEs revealed significant effects of environmental conditions on the genome integrity of animals, thereby indicating an association with their adaptability.

Keywords Peripheral lymphocytes · Adaptability · CAs · SCEs · THI · Arid · Semi-arid

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Introduction

Heat stress negatively impacts livestock performance in tropical countries during summer, which results in very important economic losses (Renaudeau et al. 2012; Koluman and Silanikove 2014). It has been predicted that the heat stress will become an increasing problem in the future due to global warming (Segnalini et al. 2013). The effect of climatic change on dairy production are both direct through effects on the livestock species themselves and indirect through effects on productivity of major crops and increased exposure to pests and pathogens (Gauly et al. 2013). These negative impacts occur in the face of increasing demands for food, which is related to increase in population (Godfray and Garnett 2014). Thus, development of adaptation strategies is necessary to minimize the negative impact of climate change and is needed to maintain food security (Knapp et al. 2014).

Genetic differences in thermotolerance at the physiological and cellular levels have been reported in many studies on *Bos indicus* and *Bos taurus* (Hansen 2004; Lacetera et al. 2006). Zebu cattle breeds are better adapted to tropical climatic conditions mainly due to the fact that these can dissipate excessive heat more effectively by sweating and are also efficient utilizers of low quality roughages compared to their crossbred

counterparts (Hansen 2004). Since India is a vast country extending between 28° 36.8′ N and 77° 12.5′ E and varied climatic conditions viz. tropical, subtropical and temperate zones, the rich animal genetic resources and the wide variety of livestock have evolved over time in these agro-climatic zones. Different species and breeds are thriving well in these conditions. The zebu breeds (Tharparkar and Kankrej) have their habitat in Rajasthan and Gujarat states (arid) where temperature extremes are observed whereas Sahiwal has a native tract in Haryana and Punjab states (semi-arid) of India.

The basic reason for better tolerance in zebu breeds could be their emergence by natural selection through generations and are therefore adapted to stressful low input environment. However, very scanty information is available about the genome integrity of zebu cattle breeds thriving in arid and semi-arid regions of India. Climatic factors like air temperature, solar radiation, relative humidity, air flow and their interactions often limit animal performance (Koluman and Silanikove 2014).

Cytogenetic techniques have been used to evaluate the effect of environmental factors on the genomic instability. The basic technique used in chromosomal abnormality assessments is sister chromatid exchange in addition to the techniques related to the detection of gaps and breaks (Iannuzzi et al. 2004). Sister chromatid exchanges and chromosome aberrations are used as cytogenetic biomarkers for analysis from peripheral lymphocytes (Ciotola et al. 2005) to evaluate the effect of different climatic conditions on genome profile of cattle under arid and semi-arid zones of northwest part of India. Sister chromatid exchange evaluation technique has been in use to detect the genome stability in humans (Seung et al. 2013) and in farm animals for example cattle (Ciotola et al. 2005), sheep (Di Meo et al. 2000), buffalo (Ahmed et al. 1998), pig (Peretti et al. 2005) and horse (Wojcik et al. 2011). There is little information about the influence of environmental factors on genome instability in zebu cattle breeds thriving in harsh climatic conditions and spread to other areas. Therefore, this study is important in order to understand the mechanisms underlying the better adaptability of zebu cattle in water-depleted and fodder-scarce regions (arid) and the breeds thriving in fodder-rich and surplus water conditions (semi-arid). This information on genome integrity in the zebu breeds is likely to further help in understanding their adaptability and sustainability in these climatic conditions. Cytogenetics in domestic animals was started in the early 1960s, and various abnormalities have also been reported in Indian cattle (Yadav 2000; Chauhan et al. 2009) and in buffaloes (Yadav et al. 1990) associated with reduced fertility or reproductive failure. However, the information related to the effect of environmental factors on the genome integrity of zebu cattle breeds is still lacking, which necessitated the need for the present study.

Materials and methods

Selection of animals

Twenty-five each of Tharparkar and Kankrej animals were selected for the study from Rajasthan University of Veterinary and Agricultural Sciences, Bikaner, Rajasthan, (arid zone) whereas 25 each of Tharparkar and Sahiwal breeds of cattle was selected from ICAR- National Dairy Research Institute, Karnal (Haryana) from semi-arid zone. All animal used in this study were of the same age (15–16 months) and sex (female). The animals were clinically healthy and free from any physical abnormalities. The animals were provided with similar managemental care and monitored closely over this period of study.

Location of study and meteorological parameters

The study was conducted at Livestock Genome Analysis Laboratory, ICAR-National Dairy Research Institute (ICAR-NDRI), Karnal, Haryana, India. The monthly average air temperature and relative humidity of arid (Bikaner) and semi-arid (Karnal) regions were recorded as climograph and presented in the form of data based on the last 30 years of climate data (Indian Meteorological Department, Pune).

Meteorological variables

Thermal stress is usually expressed in the form of temperature humidity index (THI). In terms of THI, the values of THI >72 is considered as stressful and THI >78 is considered as very severe heat stress. THI was calculated using following meteorological variables in terms of dry and wet bulb temperature and relative humidity. The THI used in this study was calculated using Thom's (1959) equation.

$$0.72(T_{db}{}^{\circ}\mathrm{C} + T_{wb}{}^{\circ}\mathrm{C}) + 40.6$$

where T_{db} is dry bulb temperature, and T_{wb} is wet bulb temperature.

The THI and weather parameters for the two climatic regions have been represented in Tables 2 and 3.

Climographs of arid and semi-arid regions

The meteorological variables, e.g. monthly average dry bulb temperature and precipitation of these locations were gathered from the Indian Meteorological Department (IMD, Pune) and presented as climographs (Figs. 4 and 5).

Collection of blood sample

Blood samples were collected in heparinized vacutainers tubes (10 mL) from jugular vein puncture and immediately

transported to laboratory and stored at 4 °C till further use for setting up cultures.

Preparation of glassware

The glassware used in tissue culture was grease free and sterilized to avoid contamination and loss of cells. It was properly cleaned, dried, autoclaved and sprayed with spirit. All the steps were carried out in laminar air flow under sterilized conditions. All glass wares were kept in chromic acid solution overnight, washed thoroughly first with running tap water and then rinsed with filtered water and finally dried in hot air oven at 150 °C for 2–4 h.

Chemicals and reagents

All chemicals and media used were of high purity and culture grade (Sigma-Aldrich, USA) unless otherwise mentioned. Heparinized vacutainers and needles were procured from Becton Dickinson (USA). Foetal bovine serum (FBS) was taken from HyClone (USA).

Preparation of culture medium

Culture medium was prepared by dissolving RPMI-1640 (2.94 g/300 mL water) in HPLC-grade water and fortified with antibiotics streptomycin (20 μg/mL) and penicillin (20 IU/mL) and phytohemagglutinin (10 μg/mL). The pH of the medium was adjusted to 7.2 with sterilized $NaHCO_3$ solution (4.4 %). The prepared medium was sterilized by filtering through millipore membrane (0.22 μm)-fixed assembly. After filtration, 20 mL serum was added to medium in laminar hood. This complete culture medium was distributed in screw capped culture bottles (30 mL) in aliquots of 6 mL each. The culture bottles were stored effectively up to 3 months under frozen condition (−20 °C) until use.

Lymphocyte culture for evaluation of chromosome aberrations

The blood samples collected were used for lymphocyte culture and preparation of chromosomes by routine method (Balakrishnan et al. 1985). Bulk culture medium was prepared, which included RPMI 1640 supplemented with 10 % foetal bovine serum, penicillin (20 IU/mL), streptomycin (20 μg/mL) and phytohemagglutinin (10 μg/mL). It was distributed into 30-mL capacity culture tubes each with 6 mL volume and stored at −20 °C till used. An aliquot of 0.5 mL blood was added per culture tube at room temperature in a clean air laminar flow hood and four replicate cultures were established for each animal. All the culture tubes were incubated for 72 h at 37.5 ± 0.5 °C.

Lymphocyte cultures for evaluation of SCEs

For revelation and evaluation of SCEs, the culture medium and conditions used were the same as described above, however, with minor modifications (Perry and Wolff, 1974). In the culture medium during incubation after 24 h of setting up of culture 10 μg/mL of 5-bromo-2′-deoxyuridine (BrdU) was added and incubated further for next 48 h.

Harvesting of the cultures

Colchicine (10 μg/mL) was added 45 min before harvesting of the cultures. Harvesting of cells was done in 15-mL centrifuge tubes and centrifugation was carried out at 1800 rpm for 15 min. Supernatant was discarded and the packed cells were treated with hypotonic salt solution (0.075 M KCl) for 8 min in a water bath at 37 °C. Subsequently, the action was stopped by an addition of 1 mL chilled fixative (methanol:acetic acid (3:1)) to each tube. The cells were washed three times with freshly prepared chilled fixative. The cell suspension was incubated overnight at −20 °C.

Cleaning of slides

The slides were dipped overnight in chromic acid solution and rinsed thoroughly in running tap water and then with filtered pure water. The slides were stored in a plastic bottle containing absolute methanol, added with 5 % HCl and kept in the refrigerator. Before use, slides were taken out, dried and cleaned thoroughly with a clean muslin cloth.

Preparation of slides for chromosome aberrations (gaps and breaks)

On a clean slide, 100 μL cell suspension was dropped from a height of 60.96 cm. Then the slides were placed on filter paper and allowed to dry at room temperature for 10–15 min. Subsequently, the slides were placed in a slide box for storage until staining was carried out.

Staining of slides for evaluation of gaps and breaks

Freshly prepared 50 mL Giemsa working solution was poured in a glass coplin jar. The required slides were kept in the coplin jar. The slides were allowed to stay in the staining solution for 30 min. Subsequently, the slides were rinsed thoroughly with distilled water and dried in the folds of filter paper. The slides were then transferred to the incubator and kept for a few hours for drying before screening or storage.

Staining of slides for evaluation of SCE's

Subsequently, the FPG (fluorescence plus Giemsa) method of chromosome staining was used. The slides were aged for 7 days then stained in 50 μg/mL Hoechst 33258 dye for 30 min. The slides were rinsed in distilled water and layered with Mcllvaine buffer (di-sodium hydrogen phosphate and citric acid, pH 7.5). Then slides were placed below a 'black blue tube light' at distance of 50.8 mm for 55 min; this step was done keeping the assembly in an incubator set at 50 °C. Slides were rinsed in Mcllvaine buffer and immersed in Giemsa solutions (4 mL stock diluted with 18 mL Mcllivaine buffer and 78 mL distilled water) for 5 min and rinsed with tap and distilled water.

Examination of Giemsa-stained and FPG-exposed metaphase-stage chromosomes

The stained slides were screened under a microscope (Leica DMRB) for evaluation of chromosome numbers, gaps, breaks, SCE and gross anomalies. For each animal, 50 good quality metaphases were examined and photographed and the data were recorded for analysis.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) (GraphPad Prism version 5.1, USA). Data of sister chromatid exchange (SCE's), chromosomal gaps and breaks are presented as mean ± SD. Data were subjected to ANOVA, and the Tukey's test was used to separate the means ($P < 0.05$) that were considered statistically significant.

Results

During the examination of conventionally Giemsa-stained metaphase plates, initially, chromosomal aberrations involving chromatid breaks and gaps were observed as shown in Fig. 1. The mean frequency of chromosomal breaks and gaps per 50 metaphase plates in Sahiwal and Tharparkar breeds of semi-arid zone were 8.72 ± 2.0, 3.52 ± 0.53 and 5.26 ± 0.01, 2.74 ± 0.17, respectively (Table 1). The mean frequency of breaks and gaps in the Tharparkar and Kankrej breeds of arid zone were 5.24 ± 1.81, 2.74 ± 0.17 and 5.24 ± 1.81, 2.5 ± 1.26, respectively (Table 1). The comparison of means revealed statistically significant differences ($P < 0.05$) between all the breeds of zebu cattle of semi-arid and arid zones. The total chromosomal aberrations in Sahiwal and Tharparkar from semi-arid region were 29.92 and 24.48, 14.72 and 15.28 % from arid zone, respectively (Table 1). Subsequently, SECs observed are shown in Fig. 3. The mean value of frequency of SCEs per cell was significantly higher in Tharparkar cattle of semi-arid region (4.72 ± 1.55) compared to that of the arid region (2.83 ± 1.01) (Figs. 1 and 3). Similarly, the frequency of SCEs was found to be 4.0 ± 1.41 in the Sahiwal breed of semi-arid region and 2.69 ± 1.12 in Kankrej breed of arid zone (Figs. 2 and 3).

Discussion

Many breeds within cattle have special features or adaptations that evolved over thousands of years of domestications in different environments (Bradley et al. 1996).The extreme variations in climatic factors like temperature, humidity and radiations are recognized as the potential hazards in the growth and production of all domestic livestock species (Gaughan et al. 1999; Hansen 2004). High ambient temperature accompanied by high air humidity causes an additional discomfort and enhances the stress level, which in turn affects the genotype as well as the phenotype of livestock species. The degree to which an animal resists change in body temperature varies with different species because of differences in their heat-regulating mechanisms (Das et al. 1999).

Chromosomes are sensitive structures vulnerable to damages from detrimental environmental factors. It is well established that numerous environmental agents (biotic and abiotic) can induce genetic alterations in human and livestock cells (Rossner et al. 2013). In the last two to three decades, animal scientists and policy makers are feeling that changes happening in climate may influence the genome profile of indigenous breeds of zebu cattle. In the present study, cytogenetic analysis revealed a significant ($P < 0.05$) instability in the genome of Sahiwal and Tharparkar animals of semi-arid zone. However, the results revealed a significantly low level ($P < 0.05$) in the chromosomal aberrations in Kankrej animals in arid zone. Nevertheless, a significant increase ($P < 0.05$) in chromosomal aberrations was observed in Tharparkar animals of semi-arid zone compared to their arid zone counterparts. The present study also revealed the a significantly higher ($P < 0.05$) frequency of SCEs per cell in Tharparkar cattle of semi-arid region (4.72 ± 1.55/cell) compared to that of the arid region (2.83 ± 1.01). Similarly, the frequency of SCEs was higher ($P < 0.05$) in the Sahiwal (4.0 ± 1.41) of semi-arid region and 2.69 ± 1.12 in Kankrej of arid zone. However, no significant differences ($P < 0.05$) were obtained amongst the cattle breeds of the semi-arid and arid zones. Till date, reports are not available on the genome integrity of zebu cattle breeds thriving in different agro-climatic zones of India. Moreover, the information about the relationship between the climatic conditions and the genome integrity of zebu cattle breeds has not been explained yet.

The previous research conducted showed correlations between the numbers of SCEs and the breed of the analysed livestock species (Iannuzzi et al. 1991). Therefore, the present

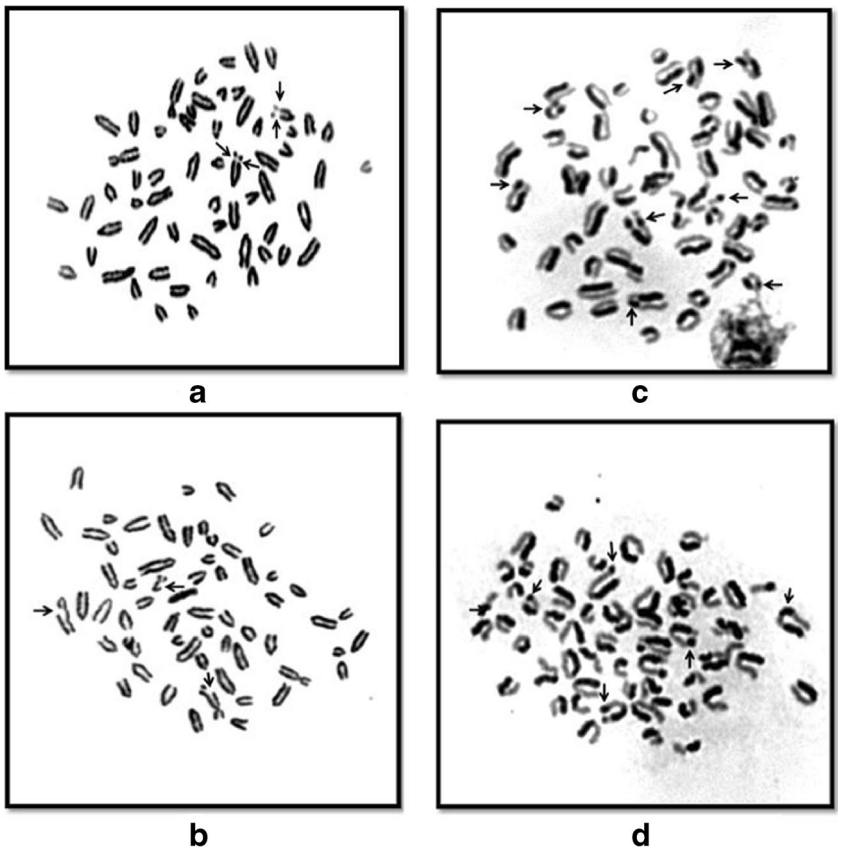

Fig. 1 Metaphase plates showing chromosome aberrations and sister chromatid exchanges (*arrows*) from two cattle breeds of semi-arid zone. **a**, **b** Chromosome aberrations in metaphase plates of Sahiwal and Tharparkar. **c**, **d** Sister chromatid exchanges in metaphase plates of Sahiwal and Tharparkar

study can be extended to the previous studies by many researchers on the genome integrity explaining the breed differences. For example, Iannuzzi et al. (1991) studied the sister chromatid exchange in chromosomes of three different cattle breeds viz. Podolian, Friesian and Romagna reared under similar conditions in Italy. Our results are in accordance with the study concluded by Iannuzzi et al. 1991, where only small differences between the mean values of SCEs/cell between the Podolian and the Friesian breeds reared under similar conditions was observed. However, in our case, a significant decrease ($P < 0.05$) in SCEs/cell was obtained between the breeds reared in different agro-climatic zones of the country (i.e. arid and semi-arid). The decrease in SCEs/cell in the Tharparkar and Kankrej breeds of arid zone could be attributed to their common descent or their common breeding tract and their superior thermo-tolerant traits. All these factors together enable specific breed to adapt to the hardy conditions of arid environment. Such factors could be probable reasons for the more adaptive capacity of Tharparkar and Kankrej to the climatic conditions of arid zone. The results obtained in the present study also suggest that the genome of Kankrej and Tharparkar cattle breeds in the arid region (Bikaner) is more stable as compared to the Tharparkar and Sahiwal breeds of semi-arid zone (Karnal) (Figs. 4 and 5). Our results also extend to the study conducted by Peretti et al. (2005) where no significant difference in SCEs/cell between endangered and indigenous pig breeds (Casertana and Italian large white) reared under similar conditions was observed. However, the results obtained in this study are contrary to those obtained by Ciotola et al. (2005) who compared their results with those of

Table 1 Chromosome aberrations (gaps and breaks) in Sahiwal, Tharparkar and Kankrej breeds of semi-arid and arid conditions

Breeds	Zone	Climatic regions	Sample size	Breaks/50 cells	Mean ± SD of breaks	*P* value (*P* < *0.05*)	Gaps/50 cells	Mean ± SD of gaps	*P* value (*P* < *0.05*)	Total aberration %
Sahiwal	Semi-arid	Karnal	25	214	8.56 ± 3.16	8.01911E−09*	160	6.4 ± 3.39	0.0001*	29.92
Tharparkar	Semi-arid	Karnal	25	218	8.72 ± 2.04		88	3.52 ± 6.29		24.48
Tharparkar	Arid	Bikaner	25	121	5.26 ± 1.76		63	2.74 ± 1.76		14.72
Kankrej	Arid	Bikaner	25	131	5.24 ± 1.81		60	2.5 ± 1.26		15.28

*Values differ in columns at P< 0.05 level of significance

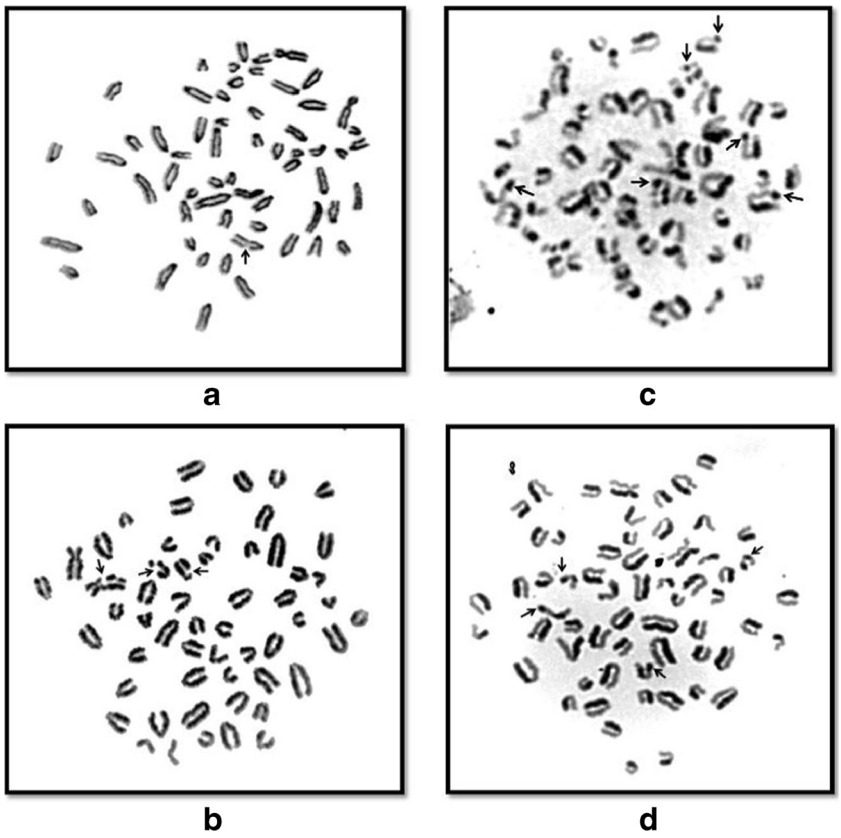

Fig. 2 Metaphase plates showing chromosome aberrations and sister chromatid exchanges (*arrows*) from two cattle breeds of arid zone. **a**, **b** CA in metaphase plates of Tharparkar and Kankrej. **c**, **d** SECs in metaphase plates of Tharparkar and Kankrej

Iannuzzi et al. (1991) and found differences in SCE frequency between the Agerolese, Podolian, Romagna and Holstein Friesien cattle breeds. Also, the current results can be extended to those of Wojcik et al. (2011) who observed a significant effect of breed/race on the SCE frequency.

The frequency of chromosomal aberrations in farm animals viz. cattle (Ciotola et al. 2005), sheep (Di Meo et al. 2000) and pigs (Peretti et al. 2005) exposed to different environmental contaminations have been reported to be higher than that of animals from normal environmental conditions. This explains the relationship between the chromosomal fragility and environmental toxicants (Das and John 1999). Lioi et al. (2004) observed an increase in the number of structural chromosome aberrations in 56 cattle raised on pastures given access to bracken fern. Similarly, the study conducted by Peretti et al. (2007) revealed the increased frequencies of both chromosome abnormalities and SCEs in two sheep flocks exposed to high dioxin levels during pasturage. There are several reports in which chromosomal fragility has been associated with the effect of teratogenic agents (Iannuzzi et al. 2004). Earlier studies on humans reported the increased chromosomal aberrations in individuals exposed to petrol and diesel exhausts and fumes (Chitra et al. 2001). The pesticides used in these studies were chlororganics and, more recently, carbamates, organophosphates and pyrethroids, which have been reported to be positive for genotoxic effects in experimental studies in bacterial and in mammalian systems (Rossner et al. 2013).

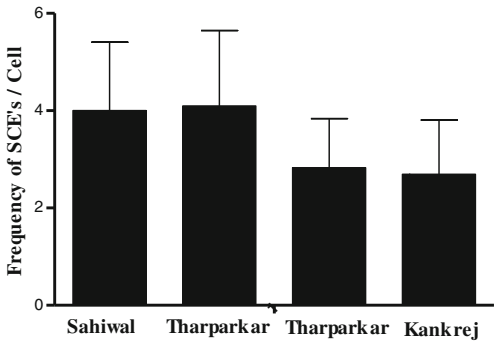

Fig. 3 SCE's frequency in the genome of Sahiwal, Tharparkar and Kankrej breeds under semi-arid and arid zone

In the present study, the lower frequency ($P < 0.05$) of CA and SCEs in the Tharpakar and Kankrej breeds of arid zone reveals their higher adaptive capacity to thrive better in the harsh climatic conditions in terms of high temperature, water scarcity and feed and fodder scarcity compared to the congenial climatic conditions of semi-arid zone. It is well known that the zebu cattle breeds have developed through a long-term natural selection and evolution over the centuries and are better adapted to withstand tropical climatic conditions in their native home tract and perform reasonably well even with low inputs of fodder availability. The results obtained also show

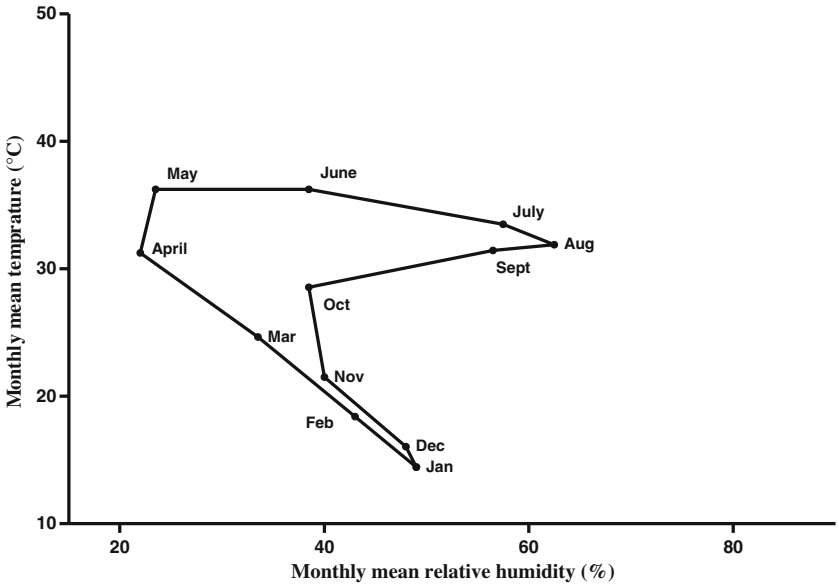

Fig. 4 Climograph based on monthly mean temperature (30 years) and monthly means relative humidity of arid zone (Bikaner)

that regional and local differences within small geographic areas might be large, and the chromosomal stability could be affected by many other factors such as soil properties, local climate, management strategies and agricultural traditions. The main climatic factors that play the major role are the high temperature and low precipitation in the arid zone compared to that in the semi-arid zone. The average THI prevailing in Karnal (semi-arid) and Bikaner (arid) has been given in Tables 2 and 3, respectively. It is quite clear that the THI in arid zone is above 75 and results indicated the stressful conditions for animals for most part of the year.

This study also supports the already existing literature information about higher adaptive capacity of indigenous cattle breeds to hot dry/hot humid conditions (Hansen, 2004). Different livestock species have different sensitivities to ambient temperature and humidity. Our results revealed that cattle breeds reared in environments that differ greatly in temperature, humidity and wind speed differ in their capacity to tolerate heat stress and become regionally adapted, thus creating sensitivity to environments that differ greatly from the un-adapted environment. This potentially decreases their chromosomal stability in un-adapted environments and usefulness across multiple regions (Hahn 1999). This is supported by the significant increase ($P < 0.05$) in the chromosomal aberrations of Tharparkar cattle reared under semi-arid climatic conditions.

High temperature, which manifests in the form of heat stress, is a major concern for livestock productivity in tropical, subtropical and arid regions of India. Thermal stress is usually expressed in the form of THI. In terms of THI, the values of

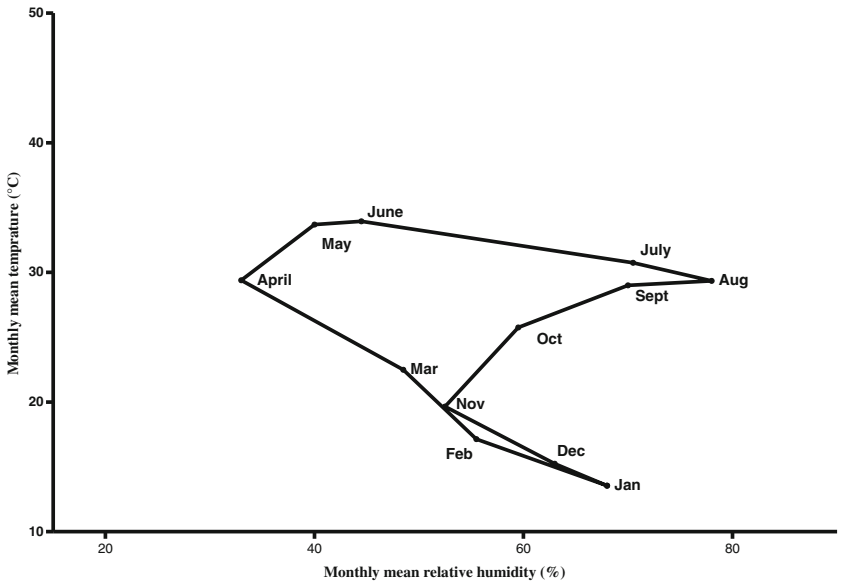

Fig. 5 Climograph based on monthly mean temperature (30 years) and monthly means relative humidity of semi-arid zone (Karnal)

Table 2 Temperature humidity index (THI) and monthly mean (±SE) weather parameters of semi-arid zone Karnal (Haryana)

Parameter/months	January	February	March	April	May	June	July	August	September	October	November	December
RH (%)	74.35 ± 1.21	65.03 ± 1.58	68.04 ± 1.51	53.76 ± 2.75	43.56 ± 2.16	60.85 ± 2.70	78.27 ± 1.86	80.72 ± 1.25	78.63 ± 1.27	73.53 ± 1.21	71.63 ± 1.61	86.80 ± 1.26
SS (h)	5.90 ± 0.50	8.07 ± 0.42	7.13 ± 0.51	9.19 ± 0.51	9.71 ± 0.43	8.44 ± 0.63	6.17 ± 0.66	6.66 ± 0.71	7.89 ± 0.53	6.22 ± 0.54	5.89 ± 0.54	2.84 ± 0.53
WS (km/h)	2.27 ± 0.40	4.20 ± 0.42	3.36 ± 0.20	4.15 ± 0.42	4.24 ± 0.32	4.85 ± 0.38	3.85 ± 0.25	4.97 ± 0.69	3.03 ± 0.21	3.15 ± 0.29	2.22 ± 0.39	2.76 ± 0.22
RF (mm)	0.42 ± 0.33	0.21 ± 0.15	0.96 ± 0.55	2.15 ± 1.03	2.48 ± 1.06	3.63 ± 2.59	5.56 ± 2.09	10.52 ± 4.08	1.02 ± 0.56	2.35 ± 1.03	0.63 ± 0.41	2.53 ± 1.67
Temp (max) (°C)	18.70 ± 0.36	21.47 ± 0.47	26.27 ± 0.33	31.64 ± 0.88	36.68 ± 0.45	36.19 ± 0.71	33.62 ± 0.46	31.88 ± 0.39	33.16 ± 0.20	27.91 ± 0.41	24.65 ± 0.57	16.25 ± 0.77
Temp (min) (°C)	4.61 ± 0.37	7.01 ± 0.54	12.27 ± 0.51	16.43 ± 0.59	20.43 ± 0.426	23.90 ± 0.40	25.93 ± 0.24	24.93 ± 0.27	23.64 ± 0.36	16.60 ± 0.34	11.51 ± 0.41	8.44 ± 0.48
THI	57.80	61.48	68.10	74.83	79.62	82.39	81.49	80.56	79.15	73.54	64.9	59.68

RH relative humidity, *SS* sunshine, *WS* wind speed, *RF* rainfall, *Temp* (*max*) temperature maximum, *Temp* (*min*) temperature minimum

Table 3 Temperature humidity index (THI) and monthly mean (±SE) weather parameters of arid zone Bikaner (Rajasthan)

Parameter/months	January	February	March	April	May	June	July	August	September	October	November	December
RH (%)	49.01 ± 2.31	52.87 ± 3.19	33.72 ± 1.66	27.15 ± 1.88	31.5 ± 3.22	45 ± 2.01	59.24 ± 1.71	55.46 ± 1.29	54.7 ± 2.03	45.04 ± 1.56	43.91 ± 1.33	55.01 ± 2.32
SS (h)	7.87 ± 0.27	7.45 ± 0.54	9.39 ± 0.28	8.69 ± 0.47	7.68 ± 0.37	7.25 ± 0.66	8.7 ± 0.36	8.37 ± 0.51	7.92 ± 0.54	9.04 ± 0.24	8.54 ± 0.22	7.70 ± 0.21
WS (km/h)	2.27 ± 0.18	3.71 ± 0.36	4.65 ± 0.25	4.65 ± 0.28	5.58 ± 0.33	8.86 ± 0.51	7.50 ± 0.40	6.87 ± 0.57	6.54 ± 0.36	2.99 ± 0.28	1.66 ± 0.17	2.17 ± 0.28
RF (mm)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.89 ± 0.42	1.48 ± 1.36	3.27 ± 2.39	2.01 ± 1.78	2.73 ± 1.50	0 ± 0	0 ± 0	0 ± 0
Temp (max) (°C)	23.33 ± 0.56	28.83 ± 0.80	34.44 ± 0.49	39.95 ± 0.59	43.23 ± 0.66	42.21 ± 0.49	38.2 ± 0.49	37.74 ± 0.28	37.88 ± 0.65	36.64 ± 0.35	31.51 ± 0.52	24.63 ± 0.45
Temp (min) (°C)	7.01 ± 0.60	14.18 ± 0.53	18.05 ± 0.43	24.40 ± 0.52	28.21 ± 0.73	28.69 ± 0.82	27.63 ± 0.49	27.78 ± 0.30	26.03 ± 0.33	20.69 ± 0.55	14.34 ± 0.50	4.75 ± 0.60
THI	57.7	62.12	69.18	75.84	82.03	84.30	83.54	82.10	80.66	74.72	66.16	59.71

RH relative humidity, *SS* sunshine, *WS* wind speed, *RF* rainfall, *Temp* (*max*) temperature maximum, *Temp* (*min*) temperature minimum

THI >72 is considered as stressful and THI >78 is considered very severe heat stress. Moreover, the prospect of global warming is encouraging renewed interest in studies related to evolution of genotypic adaptation to high temperature (IPCC 2007). Furthermore, the climate of earth has been predicted to change continuously at rates unprecedented in recent human history (IPCC 2007). Current climate models indicate an increase in temperature by 0.2 °C per decade and predict that the increase in global average surface temperature would be between 1.8 to 4 °C by 2100 (IPCC 2007). This in turn is expected to affect the production and reproduction of livestock negatively. However, the adaptive capacity of zebu cattle breeds to use poor quality feeds and fodders and to sustain extremely high temperatures makes them superior to many other breeds of livestock. It is quite possible that edaphic factors, nutrient availability, vegetation, pathogens, diseases and environmental toxicants have impact on the genome integrity in addition to temperature and precipitation.

Conclusions

The present study provided evidence about the genome integrity of Tharparkar and Kankrej breeds thriving in the hot and dry climatic conditions of arid zone. The higher genome integrity of zebu cattle breeds in arid and semi-arid zones of India might be a part of evolutionary adaptation mechanism within specific ecological niche to sustain propagation. However, the lower genome integrity observed in the Sahiwal and Tharparkar breeds of semi-arid zone compared to arid might be due to the combined interactions of air, water, soil, feed and fodder availability in this region. The observations seem to be relevant particularly in the present scenario of changing climate. The naturally evolved zebu cattle breeds can be used as invaluable genetic resources that exhibit a better adaptation to extreme climatic conditions. In view of scarcity of literature on the genome integrity of zebu cattle breeds, this information can be a baseline for future investigations. Moreover, further cytogenetic studies need to be carried out on the larger herds of zebu cattle breeds in order to understand their superior adaptability traits.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval All the animals were closely monitored and were provided similar managemental inputs during experimental period. The animals were treated following the compliance of the institute's norms for ethical treatment. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA rules laid down by government of India. Norms regarding the ethical treatment of animals during the whole operation were strictly followed.

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