CHAPTER 4
RESULTS & DISCUSSION

The present study was carried out to evaluate the genome profile of indigenous breeds of zebu cattle and riverine buffalo at different levels (genetic and physiological) under different climatic regions of northern India for assessment of adaptability in context to climate change. For evaluating the adaptability, genome integrity was of zebu cattle and buffalo was assessed by the technique of sister chromatid exchange and chromosomal aberrations. Further, to this end Real time PCR analysis of HSP70 gene super family, HSP90, HSP40, HSP10 and HSF1 during different seasons was carried out in the lymphocytes of zebu cattle and buffalo. In order to study the relationship between the adaptability and the physiology, the analysis of hormonal (Cortisol, T3 and T4) and physiological (respiration rate, pulse rate and rectal temperature) parameters has also been carried out. The climatic conditions of experimental areas i.e. Karnal, Bikaner, Hisar and Patiala districts has been presented as THI in the form of Climograph.

4.1 Chromosome profile of different breeds of cattle and buffaloes selected from different climatic regions

4.1.1 Lymphocyte culture to evaluate the genome profile

Lymphocytes were cultured for 72 hours for preparation of chromosomes to evaluate chromosome profiles of zebu and riverine buffalo under different climatic regions. For analysis of chromosome profile, overall fifty good metaphase plates were prepared and photographed for each animal and total number of cells and chromosome details have been presented in Table 4.1. Results revealed no overall numerical abnormalities in both cattle and buffalo. The metaphase plates of zebu cattle breeds have presented in figures 4.5, 4.6, 4.7, and 4.8 and metaphase plates of riverine buffalo have been presented in figures 4.9, 4.10 and 4.11 respectively. Karyotypes revealing the exact numerical abnormality in the genome of cattle breeds viz. Sahiwal and Tharparkar from semi-arid zone and Kankrej and Tharparkar from arid zone
have been presented in figures 4.12, 4.13, 4.14 and 4.15 respectively. Similarly, the karyotype of riverine buffalo have been presented in figures 4.16, 4.17 and 4.18 using cytovision software of Lieca (version 7.2).

**Table 4.1 Chromosomal profile of breeds of riverine buffalo and zebu cattle under semi-arid and arid regions**

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Climatic regions</th>
<th>Climatic region</th>
<th>N</th>
<th>No. of cells</th>
<th>No. of chromosome</th>
<th>2n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffaloes</td>
<td>Hisar</td>
<td>Semi-arid</td>
<td>25</td>
<td>1250</td>
<td>62500</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Karnal</td>
<td></td>
<td>25</td>
<td>1250</td>
<td>62500</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Nabha</td>
<td></td>
<td>25</td>
<td>1250</td>
<td>62500</td>
<td>50</td>
</tr>
<tr>
<td>Sahiwal</td>
<td>Karnal</td>
<td></td>
<td>25</td>
<td>1250</td>
<td>75000</td>
<td>60</td>
</tr>
<tr>
<td>Tharparkar</td>
<td></td>
<td>Arid</td>
<td>25</td>
<td>1250</td>
<td>75000</td>
<td>60</td>
</tr>
<tr>
<td>Tharparkar</td>
<td>Bikaner</td>
<td>Arid</td>
<td>25</td>
<td>1250</td>
<td>75000</td>
<td>60</td>
</tr>
<tr>
<td>Kankrej</td>
<td></td>
<td></td>
<td>25</td>
<td>1250</td>
<td>75000</td>
<td>60</td>
</tr>
</tbody>
</table>

4.2 To evaluate chromosomal aberrations and sister chromatid exchanges (SCEs) in chromosomes of animals selected from different climatic regions

4.2.1 Chromosome aberration in the genome of zebu cattle

During the examination of conventionally Giemsa stained metaphases, initially chromosomal aberrations involving chromatid breaks and gaps were observed as shown in figures 4.20, 4.21, 4.22 and 4.23. The mean frequency of breaks and gaps per 50 cells in Sahiwal and Tharparkar breeds of semi-arid zone was 8.72±2.0, 3.52±0.53 and 5.26±0.01, 2.74±0.17 respectively (table 4.3). 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. 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 the zebu cattle (Sahiwal and Tharparkar) from semi-arid region were 29.92 and 24.48; 14.72 and 15.28% from arid zone, respectively (table 4.3). 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) (figure 4.19, 4.25 and 4.26). 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 (figure 4.19, 4.24 and 4.27).

### 4.2.2 Chromosome aberrations and sister chromatid exchanges in the genome of riverine buffalo breeds

The mean frequency of chromosomal aberrations including gaps and breaks in each breed of buffalo are shown in figure 4.28. The frequency of gaps in Murrah buffalo from Hisar and Karnal was 12.48±4.12 and 10.86±3.65 and respectively. The range of gaps was 3-14 and 3-16 per 50 cells in the Murrah buffalo from Hisar and Karnal regions respectively. The mean frequency of breaks in Murrah buffalo from Hisar and Karnal regions was 5.96±1.92, and 5.44±2.72 respectively. Results indicated that the range of breaks in Murrah buffalo from Hisar and Karnal was 0-6 and 0-7 per 50 cells respectively (figure 4.28). Similarly, the frequency of gaps in Nili Ravi buffalo breed from Patiala region was 7.0±2.27 ranging between 1-13 per 50 cells. Also, the mean frequency of breaks in Nili Ravi buffalo was 3.1±1.97. The range of gaps was 1-6 per 50 cells in this breed of buffalo (figure 4.28).

In the present study, the frequency of sister frequency chromatid (SCE) in Murrah and Nili-Ravi buffalo from Hisar, Karnal and Patiala regions has also been studied. The mean frequency of sister chromatid exchange (SCE) in each breed of buffalo is shown in figure 4.29. The frequency of SCE in Murrah buffalo from Hisar and Karnal was 6.64±2.21 and 6.26±1.92 respectively. The range of SCEs was 1-9 and 2-9 per cell in Murrah buffalo from Hisar and Karnal regions respectively. The frequency of SCE in Nili Ravi buffalo from
Results & Discussion

Patiala region was 3.52±1.87 ranging between 1 to 8. Comparison of means revealed significantly higher \((P \leq 0.01)\) frequency of CAs and SCEs in the Murrah buffalo from Karnal region compared to the Hisar region. Statistical analysis revealed significant differences \((P \leq 0.01)\) between mean frequency of SCEs in Murrah and Nili-Ravi breeds of buffalo from all the three regions. The metaphase plates showing the CAs and the SCE in the genome of Murrah and Nili Ravi buffalo breeds from Hisar, Karnal and Patiala region have been represented in figures 4.30-4.35 respectively.

4.2.3. Climatic conditions

In order to compare the climatic conditions of the four regions (Bikaner, Hisar, Karnal and Patiala), information on the monthly average temperature and precipitation of these locations was gathered from Indian meteorological department (IMD, Pune), and climographs for these regions were prepared based on this information (figures 4.1, 4.2, 4.3 and 4.4). The temperature humidity index (THI) for these climatic regions has been represented in table 4.2. Moreover, the agro-climatic zones of India have been represented in the Table 2.1.

Table 4.2 Climatic conditions of experimental regions

<table>
<thead>
<tr>
<th>Climatic regions</th>
<th>Annual Maximum Temp. °C</th>
<th>Average maximum temp. during month of June °C</th>
<th>Annual Minimum °C</th>
<th>Average minimum temp. during month of January °C</th>
<th>Rainfall mm</th>
<th>THI</th>
<th>Geographical location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karnal</td>
<td>30.4</td>
<td>38.9</td>
<td>17.5</td>
<td>7.1</td>
<td>56.32</td>
<td>71.95</td>
<td>29 50° N 76 31° E</td>
</tr>
<tr>
<td>Amritsar</td>
<td>30.5</td>
<td>39.8</td>
<td>15.5</td>
<td>3.9</td>
<td>59.86</td>
<td>71.95</td>
<td>30.32°N 76.40°E</td>
</tr>
<tr>
<td>Hisar</td>
<td>32.6</td>
<td>41.2</td>
<td>17.7</td>
<td>5.8</td>
<td>40.89</td>
<td>71.96</td>
<td>29.09°N 75.43°E</td>
</tr>
<tr>
<td>Bikaner</td>
<td>33.6</td>
<td>41.8</td>
<td>19.1</td>
<td>6.7</td>
<td>25.84</td>
<td>73.14</td>
<td>28.01° N 73.19° E</td>
</tr>
</tbody>
</table>

(Source Indian Metrological Department, IMD)
Table No.4.3 Chromosomal aberrations of breeds of zebu cattle under arid and semi-arid regions

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Climatic regions</th>
<th>Sample size</th>
<th>Total breaks/ 50 cells</th>
<th>Mean ± S.D. of breaks</th>
<th>Total gaps/ 50 cells</th>
<th>Mean ± S.D. of gaps</th>
<th>P Value (P&lt;0.05)</th>
<th>Total aberration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahiwal</td>
<td>Semi</td>
<td>25</td>
<td>214</td>
<td>8.56±3.16</td>
<td>160</td>
<td>6.4±3.39</td>
<td>0.01911E-09</td>
<td>29.92</td>
</tr>
<tr>
<td>Tharparkar</td>
<td>arid</td>
<td>25</td>
<td>218</td>
<td>8.72±2.04</td>
<td>88</td>
<td>3.52±6.29</td>
<td>0.0001</td>
<td>24.48</td>
</tr>
<tr>
<td>Tharparkar</td>
<td>arid</td>
<td>25</td>
<td>121</td>
<td>5.26±1.76</td>
<td>63</td>
<td>2.74±1.76</td>
<td>0.0001</td>
<td>14.72</td>
</tr>
<tr>
<td>Kankraj</td>
<td></td>
<td>25</td>
<td>131</td>
<td>5.24±1.81</td>
<td>60</td>
<td>2.5±1.26</td>
<td>0.0001</td>
<td>15.28</td>
</tr>
</tbody>
</table>
4.3 To evaluate the major types of genes of \emph{HSP-70} family using different molecular techniques

4.3.1 Gene expression level in cattle and buffalo during different seasons

4.3.2 Isolation of RNA

Total RNA was isolated from the lymphocytes in of Sahiwal, Tharparkar and Murrah buffalo groups and the detailed procedure has been described in Materials and Methods (section 3.9.1). The integrity of RNA was checked by running on 1.5% agarose gel. Two distinct intact bands on gel represented two subunits of RNA, one for 18S and the other one for 28S respectively (figure 4.48)

4.3.3 Concentration and quality of total RNA isolated from lymphocyte

The concentration of total RNA was within the range of 35 to 600ng/µl with good quality. An optical density (OD) 260/280 ration greater than 1.9 was usually accepted for RNA quality under this study (figure 4.47).

4.3.4 Optimization of real time PCR conditions for \emph{HSP70} super family, \emph{HSP90}, \emph{HSP40}, \emph{HSP10}, \emph{HSF1} and housekeeping gene primers

Before performing real time PCR, optimization of real time PCR conditions and primer matrix was done for all the primers of \emph{HSP70} family , \emph{HSP90}, \emph{HSP60}, \emph{HSP40}, \emph{HSP10}, \emph{HSF1} and housekeeping gene (\emph{GAPDH}). The products obtained after amplification were analyzed on 2% agarose gel electrophoresis (figure 4.47). The presence of single bands of expected sizes for all the above mentioned genes and housekeeping genes signified that the primers were highly specific to their target sequences in oocytes studied under present conditions.

4.3.5 Real time PCR analysis

To determine the specificity of the PCR reaction (a single specific peak and to detect primer/dimer formation), amplification plots and dissociation curve was generated after completion of amplification, to check unspecific binding and primer dimer formation. Single peak in all experiments during Real-Time PCR signified that the primers were highly specific to the target and there was not any dimer formation (figures 4.50, 4.52, 4.54, 4.56 and 4.58).
4.3.6 Relative expression profiling of all HSPs gene

The results of m-RNA expression of HSP gene (70.1, 70.2, 70.8, HSP90, HSP40, HSP 10 and HSF1) in lymphocytes of Sahiwal, Tharparkar and Murrah buffalo during different seasons (figures 4.49, 4.51, 4.53, 4.55, 4.57, 4.59, 4.60) and statistical analysis have been presented in table no. 4.4, 4.5 and 4.6.

4.3.7 Relative expression of HSP70.1

The results on relative expression of HSP70.1 in Tharparkar and Sahiwal cattle and Murrah buffalo have been represented in figure 4.49 and table 4.4, 4.5 and 4.6. Results revealed that the expression of HSP70.1 in Tharparkar and Sahiwal cattle and Murrah buffaloes increased significantly ($P \leq 0.001$) during summer season and winter compared to control (thermoneutral). However, the magnitude of increase ($P \leq 0.01$) was higher during summer season compared to the winter season. The increase in HSP70.1 gene expression during summer season was 9.70±1.54, 10.65±0.95 and 14.48±2.31 folds in Tharparkar, Sahiwal and Murrah buffaloes respectively compared to the control group. Similarly, the expression of mRNA increased by 4.23±1.02, 4.57±1.23 and 5.60±1.98 folds during winter season in Tharparkar, Sahiwal and Murrah buffaloes respectively compared to control. Results revealed a significantly higher ($P \leq 0.01$) expression of HSP70.1 in cattle and buffalo breeds during summer season compared to winter control group (thermoneutral).

4.3.8 Relative expression of HSP70.2

The results on relative expression of HSP70.2 in Tharparkar and Sahiwal cattle and Murrah buffalo have been presented in figure 4.51 and table 4.4, 4.5 and 4.6. Results revealed that the expression of HSP70.1 in Tharparkar and Sahiwal cattle and Murrah buffaloes cattle increased significantly ($P \leq 0.001$) during summer season and winter compared to control (thermoneutral). The increase in HSP70.2 gene expression during summer season was 9.51±2.31, 14.81±3.01 and 13.55±1.95 folds in Tharparkar, Sahiwal and Murrah compared to the control group. The HSP70.2 gene expression during winter season increased by 3.33±1.20, 4.89±0.54 and
RESULTS & DISCUSSION

5.18±1.20 folds in Tharparkar and Sahiwal cattle and Murrah buffaloes respectively. The relative HSP70.2 gene expression in Tharparkar and Sahiwal cattle and Murrah buffalo increased to significantly \((P≤0.001)\) during summer season compared to winter and control group (thermoneutral).

4.3.9 Relative expression of HSP70.8

The results on relative expression of HSP70.8 in Tharparkar and Sahiwal and Murrah buffalo have been presented in figure 4.53 and table 4.4, 4.5 and 4.6. The fold change in HSP70.8 gene expression was 5.56±0.61, 5.17±0.15 and 6.01±1.05 respectively in Tharparkar and Sahiwal cattle and Murrah buffaloes during summer season. The HSP70.8 gene expression during winter season was 4.81±0.98, 4.69±0.87 and 4.40±0.52 folds respectively in Tharparkar and Sahiwal cattle and Murrah buffaloes. The relative HSP70.8 gene expression increased significantly \((P>0.01)\) during summer and winter season compared to control group in all the three categories of animals.

4.3.10 Relative expression of HSP90

The results on relative expression of HSP90 in Tharparkar and Sahiwal cattle and Murrah buffalo have been presented in figure 4.55 and table 4.4, 4.5 and 4.6. The fold change in HSP90 gene expression during summer season was 2.87±0.02, 3.77±0.23 and 2.53±0.68 folds in Tharparkar and Sahiwal cattle and Murrah buffaloes respectively. The HSP90 gene expression during winter season was 1.62±0.12, 1.67±0.02 and 1.27±0.08 folds in Tharparkar and Sahiwal cattle and Murrah buffaloes respectively. The relative HSP90 gene expression increased significantly \((P≤0.05)\) during summer season compared to control group in Tharparkar and Sahiwal and Murrah buffalo. The results showing that the fold change in HSP 90 expression during winter and summer seasons between the breeds of cattle and buffalo was non- significant.

4.3.11 Relative expression of HSP40

The results on relative expression of HSP40 in Tharparkar and Sahiwal cattle and Murrah buffalo have been presented in figure 4.57 and statistical analysis in table 4.4, 4.5 and 4.6. The fold change in HSP40 gene expression
during summer season was 6.58±1.24, 7.64±0.83 and 4.87±1.02 in Tharparkar, Sahiwal and Murrah buffaloes respectively. The *HSP40* gene expression during winter season was 1.21±0.61, 1.51±0.21 and 1.63±0.02 folds in Tharparkar, Sahiwal and Murrah buffaloes respectively (figure 4.57). The relative *HSP40* gene expression increased significantly (*P* ≤ 0.001) during summer season compared to control group in Tharparkar, Sahiwal and Murrah buffalo under tropical climate condition.

4.3.12 Relative expression of *HSP10*

The results on relative expression of *HSP10* in Tharparkar and Sahiwal cattle and Murrah buffalo have been presented in figure 4.59 and table 4.4, 4.5 and 4.6. The fold increase in *HSP10* gene expression during summer season was 6.59±1.05, 7.02±0.51 and 6.25±0.12 respectively in Tharparkar and Sahiwal cattle and Murrah buffaloes compared to the control group (thermoneutral). The *HSP10* gene expression during winter season was 2.54±0.02, 3.09±0.65 and 3.27±0.15 folds in Tharparkar, Sahiwal and Murrah buffaloes respectively. Results revealed the significantly higher (*P*≤0.001) expression of *HSP10* during summer season in Tharparkar, Sahiwal and Murrah animals compared to winter season and control group (thermoneutral).

4.3.13 Relative expression of *HSF1*

The results on relative expression of *HSF1* in Tharparkar and Sahiwal cattle and Murrah buffalo have been presented in figure 4.60 and tables 4.4, 4.5 and 4.6. The increase in the mRNA abundance of *HSF1* gene during summer season was 4.38±1.05, 4.53±0.95 and 5.68±0.45 folds respectively in Tharparkar and Sahiwal cattle and Murrah buffaloes respectively. The *HSF1* gene expression during winter season was 1.04±0.02, 1.25±0.32 and 1.00±0.84 folds in Tharparkar and Sahiwal cattle and Murrah buffaloes respectively. Results depicted the significantly higher expression of *HSF1* during summer and winter compared to the control group (thermoneutral). However, the magnitude of induction was significantly higher (*P*≤0.001) during season compared to winter.
Table 4.4 Relative HSPs gene expression level (2^−ΔΔCT) measured in lymphocytes of Tharparkar cattle

<table>
<thead>
<tr>
<th>Genes</th>
<th>Seasons</th>
<th>70.1</th>
<th>70.2</th>
<th>70.8</th>
<th>HSP90</th>
<th>HSP40</th>
<th>HSP10</th>
<th>HSF1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>4.23±1.02</td>
<td>3.33±1.20</td>
<td>4.81±0.98</td>
<td>1.62±0.12</td>
<td>1.21±0.61</td>
<td>2.54±0.02</td>
<td>1.04±0.02</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>9.70±1.54***</td>
<td>9.51±1.31***</td>
<td>5.56±0.61</td>
<td>2.87±0.02</td>
<td>6.58±1.24***</td>
<td>6.59±1.05**</td>
<td>4.38±1.05</td>
</tr>
</tbody>
</table>

* P ≤ 0.05    ** P ≤ 0.01    *** P ≤ 0.001

Table 4.5 Relative HSPs gene expression level (2^−ΔΔCT) measured in lymphocytes of Sahiwal cattle

<table>
<thead>
<tr>
<th>Genes</th>
<th>Seasons</th>
<th>70.1</th>
<th>70.2</th>
<th>70.8</th>
<th>HSP90</th>
<th>HSP40</th>
<th>HSP10</th>
<th>HSF1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>4.57±1.23</td>
<td>4.89±0.54</td>
<td>4.69±0.87</td>
<td>1.67±0.02</td>
<td>1.51±0.21</td>
<td>3.09±0.65</td>
<td>1.25±0.32</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>10.65±0.95***</td>
<td>14.81±1.01***</td>
<td>5.17±0.15</td>
<td>3.77±0.23</td>
<td>7.64±0.83**</td>
<td>7.02±0.51**</td>
<td>4.53±0.95</td>
</tr>
</tbody>
</table>

* P ≤ 0.05    ** P ≤ 0.01    *** P ≤ 0.001

Table 4.6 Relative HSPs gene expression level (2^−ΔΔCT) measured in lymphocytes of Murrah buffalo

<table>
<thead>
<tr>
<th>Genes</th>
<th>Seasons</th>
<th>70.1</th>
<th>70.2</th>
<th>70.8</th>
<th>HSP90</th>
<th>HSP40</th>
<th>HSP10</th>
<th>HSF1</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>5.60±1.98</td>
<td>5.18±1.20</td>
<td>4.40±0.52</td>
<td>1.27±0.08</td>
<td>1.63±0.02</td>
<td>3.27±0.15</td>
<td>1.00±0.84</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>14.48±2.31***</td>
<td>13.55±1.95***</td>
<td>6.01±1.05</td>
<td>2.53±0.68</td>
<td>4.87±1.02**</td>
<td>6.25±0.12**</td>
<td>5.68±0.45</td>
</tr>
</tbody>
</table>

* P ≤ 0.05    ** P ≤ 0.01    *** P ≤ 0.001
4.3.14 Amplification of *HSP70* gene using conventional PCR

DNA samples were isolated from Tharparkar, Sahiwal and Kankrej breeds of cattle (semi-arid and arid regions) and Murrah and Nili-Ravi buffalo (semi-arid region). *HSP70* gene was amplified with the customized primers using DNA as the template with conventional PCR. The amplified products were electrophoresed on 2% Agarose gel and visualized under gel-documentation system. The amplified gene products of each breed of cattle and buffalo have been represented in figures 4.36-4.41.

4.3.15 Sequencing of amplified PCR products of *HSP70* gene

The amplified gene products for *HSP70* gene were purified and sequenced. The sequence of *HSP70* gene was aligned for homology using the BLAST tool of NCBI. After validation, the aligned sequence was submitted to the gene bank and the resultant accession no. has been represented in table 4.7. The comparison of *HSP70* sequence in each breed of cattle and buffalo (Sahiwal, Tharparkar, Kankrej, Murrah and Nili-Ravi) revealed individual nucleotide differences as represented in Figures 4.42-4.46.

**Table 4.7 The accession number for *HSP70* gene (5’ UTR) of three breeds of cattle and two riverine buffalo**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Seq No.</th>
<th>Accession No.</th>
<th>Breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BankIt1730967 Seq1</td>
<td>KJ917776</td>
<td>Murrah</td>
</tr>
<tr>
<td>2.</td>
<td>BankIt1730970 Seq1</td>
<td>KJ917777</td>
<td>Nili-Ravi</td>
</tr>
<tr>
<td>3.</td>
<td>BankIt1730973 Seq1</td>
<td>KJ917778</td>
<td>Kankrej</td>
</tr>
<tr>
<td>4.</td>
<td>BankIt1730975 Seq1</td>
<td>KJ917779</td>
<td>Kankrej</td>
</tr>
<tr>
<td>5.</td>
<td>BankIt1730976 Seq1</td>
<td>KJ917780</td>
<td>Tharparkar</td>
</tr>
</tbody>
</table>
4.4 To correlate chromosomal changes and genes of HSP-70 family with physiological factors in cattle and buffaloes

4.4.1 Physiological parameters

Results observed for physiological parameters viz. respiration rate (RR), pulse rate (PR), rectal temperature (RT) and body temperatures in cattle (Tharparkar, Sahiwal and Kankrej) and buffalo (Murrah and Nili-Ravi) breeds from semi-arid (Karnal, Hisar and Patiala) and arid (Bikaner) have been presented in table 4.8 and figures 4.61, 4.62 and 4.68.

Table 4.8 Physiological responses of different breeds of cattle and buffalo

<table>
<thead>
<tr>
<th>Breeds</th>
<th>RR/min</th>
<th>PR/min</th>
<th>RT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± SD</td>
<td>Mean± SD</td>
<td>Mean± SD</td>
</tr>
<tr>
<td>Sahiwal</td>
<td>31.33±3.54</td>
<td>50.66±3.03</td>
<td>39.13±0.38</td>
</tr>
<tr>
<td>Tharparkar</td>
<td>30.0±3.16</td>
<td>53.0±2.91</td>
<td>39.33±0.36</td>
</tr>
<tr>
<td>Tharparkar-arid</td>
<td>27±2.31</td>
<td>42±1.98</td>
<td>37.85±0.56</td>
</tr>
<tr>
<td>Kankrej</td>
<td>26.0±2.84</td>
<td>45.0±2.14</td>
<td>38.22±1.37</td>
</tr>
<tr>
<td>Murrah</td>
<td>30.12±1.36</td>
<td>60.23±2.36</td>
<td>38.33±0.51</td>
</tr>
<tr>
<td>Murrah-KNL</td>
<td>26.33±2.73</td>
<td>58.38±3.99</td>
<td>39.22±0.43</td>
</tr>
<tr>
<td>Nili-Ravi</td>
<td>19.90±2.54</td>
<td>49.33±2.16</td>
<td>37.99±0.72</td>
</tr>
</tbody>
</table>

4.4.2 Respiration rate (RR)

The average normal respiration rate in Sahiwal breed of semi-arid zone was 50.66±3.03 /min. For Tharparkar of arid and semi-arid zone, PR was 42±1.98 and 30.0±3.16 respectively. The normal respiration rate in Kankrej of arid zone was 26.0±2.84. Similarly, the average normal respiration rate in Murrah buffalo breed semi-arid zone (Karnal and Hisar) was 58.38±3.99 and 30.12±1.36 breaths/min respectively. The normal respiration rate in Nili-Ravi
Results & Discussion

The average normal pulse rate in Sahiwal was 31.33±3.54 breaths/min. For Tharparkar of arid and semi-arid zone, PR was 42±1.98 and 53.0±2.91 respectively. The normal pulse rate in Kankrej of arid zone was 45.0±2.14. Similarly, the normal pulse rate in Murrah buffalo breed semi-arid zone (Karnal and Hisar) was 26.33±2.73 and 60.23±2.36 respectively. The normal pulse rate recorded in Nili-Ravi breed of buffalo was 49.33±2.16 pulses/min. Results representing the average normal pulse rate have been presented in table 4.8 and figures 4.64 and 4.67.

4.4.4 Rectal temperature

The average normal rectal temperature in Sahiwal breed of semi-arid zone was 39.13±0.38°C. For Tharparkar of arid and semi-arid zone, RT was 37.85±0.56° and 39.33±0.36°C respectively. The normal rectal temperature in Kankrej of arid zone was 38.22±1.37°C. Similarly, the average normal rectal temperature in Murrah buffalo breed semi-arid zone (Karnal and Hisar) was 39.22±0.43° and 38.33±0.51°C respectively. The normal rectal temperature in Nili Ravi breed of buffalo was 37.99±0.72 °C. Results representing the average normal rectal temperature have been presented in table 4.10 and figures 4.65 and 4.67.

4.4.5 Hormonal Parameters

In a separate set of experiments, hormonal (Cortisol, T3 and T4) parameters were assessed in the plasma collected from the zebu cattle (Sahiwal, Tharparkar and Kankrej) and buffalo breeds (Murrah and Nili-Ravi) of arid and semi-arid zones. Results on the hormonal parameters are represented in figures 4.69-4.73.
4.4.6 Cortisol

The average plasma cortisol (ng/ml) level was 5.34±1.86 and 14.0±2.62, in the Sahiwal and Tharpakar from semi-arid zone. The level of cortisol in Tharparkar of arid and Kankrej breeds from arid zone was 0.72±.53 and 0.73±.32 respectively (figure 4.70). Similarly the level of cortisol in Murrah buffalo breed from the Karnal and Hisar region (semi-arid) was 2.82±0.58 and 3.11±1.52 ng/ml respectively. The level of cortisol observed for Nili-Ravi breed of buffalo was 1.05±0.66 ng/ml (figure 4.71). Results indicated significantly higher \((P<0.001)\) cortisol level in the Tharparkar of semi-arid zone compared to that of the Sahiwal breed of cattle. No significant difference in the cortisol level was observed between Tharparkar and Kankrej breeds of cattle from the arid zone. Significantly higher \((P<0.01)\) level of cortisol was observed in the plasma of Murrah buffalo from Hisar compared to that Karnal region. The level of cortisol was significantly lower \((P<0.01)\) in the Nili-Ravi breed of buffalo compared to the Murrah breed of arid and semi-arid zone.

4.4.7 T\(_3\) and T\(_4\) level in plasma

The average plasma T\(_3\) level was 2.89±1.29 and 1.13±1.37 in the Sahiwal and Tharparkar from semi-arid zone. For the Tharparkar and Kankrej breeds of arid zone, the T\(_3\) level was 1.60±2.47 and 3.81±1.06 ng/ml respectively. The average plasma T\(_4\) (ng/ml) level was 0.99±1.36, 7.31±2.06, 1.85±1.57 and 1.56±1.19 in Sahiwal, Tharpakar of semi-arid and Tharparkar and Kankrej breeds of arid zone respectively (figure 4.72). Similarly, the level of T\(_3\) in the Murrah and Nili-Ravi breeds of buffalo from Karnal, Hisar and Nabha (semi-arid) was 1.22±0.230, 89±0.16 and 1.27±0.31 ng/ml respectively. The level of T\(_4\) in the plasma of the Murrah and Nili-Ravi breeds of buffalo from Karnal, Hisar and Nabha (semi-arid) was 37±2.095, 39±1.696 and 5.60±1.27 respectively (figure 7.73).

Statistical analysis revealed no significant variations in the T\(_3\) level between the cattle breeds of arid and semiarid zone. But a significant increase \((P<0.001)\) in the level of T\(_4\) was observed for the Tharparkar of semiarid zone compared to the arid zone. Also, a significant increase \((P<0.001)\) in the T\(_4\) level
was observed between the Tharparkar of semiarid zone compared to the Sahiwal cattle of the same zone. Comparison of means revealed non-significant differences between the T₃ and T₄ levels in the plasma of Murrah and Nili-Ravi buffalo breeds from all the three locations (Karnal, Hisar and Nabha) of semi-arid zone.

**Discussion**

In the present study, genome integrity of zebu cattle and buffalo breeds in different regions of arid (Bikaner) and semiarid (Karnal, Hisar and Nabha) zones in India was studied. The aim of the present study was to evaluate the genome integrity of zebu cattle and buffalo breeds using the cytogenetic analysis and the expression pattern of heat shock protein genes that are reported to be induced under the tropical 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. Moreover, the prospect of global warming (IPCC, 2007) is encouraging renewed interest in studies related to evolution of genotypic adaptation to high temperature. 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 °C to 4 °C by 2100 (IPCC, 2007). This would in turn affect the production and reproduction of livestock negatively. Also, the physiological and hormonal parameters were studied in order to ascertain the relationship between the physiological behavior and genome integrity. The overall objective of this study was to evaluate the effect of respective native and adaptive breeding tract on the genome integrity of zebu cattle and buffalo breeds.

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 (Finch, 1986; 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
RESULTS & DISCUSSION

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). In the present study, cytogenetic analysis in cattle revealed significant \((P<0.05)\) instability in the genome of Sahiwal and Tharparkar animals of semiarid zone. However, the results revealed significantly lower \((P<0.05)\) frequency of chromosomal aberrations in Kankrej animals in arid zone. Significant increase \((P<0.05)\) in the chromosomal aberrations was observed in Tharparkar animals of semi-arid zone compared to their arid zone counter parts.

The present study also revealed the significantly higher \((P<0.05)\) frequency of SCEs per cell in Tharparkar cattle of semi-arid region \((4.72 \pm 1.55\) per cell) compared to that of the arid region \((2.83 \pm 1.01)\). Similarly, the frequency of SCEs was higher \((P <0.05)\) in the Sahiwal \((4.0 \pm 1.41)\) of semi-arid region and \(2.69 \pm 1.12\) 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, very less information is 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 revealed correlations between the number of SCEs and the breed of the analysed livestock species (Iannuzzi et al., 1991). Therefore, the present 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 this study, 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, significant decrease \((P<0.05)\) in SCEs/cell between the breeds reared in different agro-climatic zones of the country (i.e. arid and semi-arid) was obtained. 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 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 this 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). Di Berardino and Shoffner (1979) 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 humans (Chaganti et al., 1974), and farm animals viz. cattle (Ciotola et al., 2005), buffalo (Iannuzzi et al., 1988) sheep (Di Meo et al., 2000), and pigs (Rubes, 1987; Peretti et al., 2006) 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 (Rubes, 1987; Bires et al., 1993.; Parada and Jaszczak, 1993; Ghaffar et al., 1994; Soldatovic et al., 1994; Sivikova and Dianovsky, 1995). 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 chromosomal 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 (Postiglioni et al., 1996; Iannuzzi et al., 2004). Earlier studies on humans reported the increased chromosomal aberrations in individuals exposed to petrol and diesel exhausts and fumes (Sobti and Bhardwaj, 1993; Khalil, 1995; Knudsen et al., 1999; 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 (Garrett et al., 1986; Bolognesi and Merlo, 1995; Dearfield et al., 1999).

In the present study, the lower frequency \((P<0.05)\) of CA and SCEs in the Tharpakar and Kankrej breeds of arid zone reveal their higher adaptive capacity to thrive better in the harsh climatic conditions in terms of high temperature, water scarcity, 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 therefore, 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 in this study also show 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 table 4.1. It is quite clear that the THI in arid zone is above 75 and results indicated the stressful conditions for animals. Despite this stressful THI for most part of the year, the zebu cattle breeds are performing well which may be attributed to the superior genome integrity as revealed by this study.

This study also supports the already existing information (Hansen, 2004) about higher adaptive capacity of indigenous cattle breeds to hot dry/ hot humid conditions. 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 (Young, 1983; Hahn, 1999). This is supported by the significant increase \((P<0.05)\) in the chromosomal aberrations of Tharparkar cattle reared under semiarid climatic conditions.

Therefore, the present study reveals that the Zebu cattle have higher adaptability, which suits them better in tropical climate and also makes them an efficient utilizer of low quality roughages. The zebu breeds viz. Tharparkar, Sahiwal and Kankrej have their habitat in Rajasthan and Gujarat (arid and semi-arid) where temperature extremes are observed. Some of the breeds have been moved from their native tract to other places e.g. Karnal. 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 and fifteen agro-climatic regions, the rich animal genetic resources and the wide variety of livestock have evolved over time.

Also, the cytogenetic analysis in buffalo breeds revealed significant \((P \leq 0.01)\) instability in the genome of Murrah compared to Nili-Ravi buffalo breed in their respective breeding tracts. The variations in the number of CAs and SCEs between the two buffalo breeds could be due the different intensity of selection, the prevailing demographic factors, management strategies and agricultural traditions. Therefore, this better capacity in Nili-Ravi as revealed by the lower mean frequency of CAs and SCEs has been developed through long-term natural selection and evolution over the centuries. The results obtained in this study can be compared to the previous studies by many researchers. Iannuzzi et al. (1988) studied SCE in riverine buffalo genome reared in southern Italy. Their results indicated a mean frequency value of 8.8±3.4 SCE/cell which is considerably higher than the mean values of 6.64±2.21 (Murrah) and 3.52±1.87 (Nili-Ravi) observed in the present study. The present values are close to the results reported in Bhadawari buffaloes (5.56 per cell) and Murrah buffaloes (3.66 per cell) (Vijh et al., 1991; Vijh et al., 1995). However, the mean frequency of SCE/cell obtained in the present study for both the buffalo breeds is lower than Surti (14.05 ± 0.12) (Murali et al., 1998) and Toda (7.8±0.23) breed of buffalo (Murali et al., 2009).
The present results are close to the previous results reported in Bhadawari (5.56 per cell) and Murrah buffaloes (3.66 per cell) (Vijh et al., 1991; Vijh et al., 1995). However, the mean frequency of SCE/cell obtained in the present study for both the buffalo breeds is lower than Surti (14.05 ± 0.12) (Murali et al., 1998) and Toda (7.8±0.23) breed of buffalo (Murali et al., 2009).

The results obtained here can also be extended to previous studies carried out in Egyptian buffaloes by Ahmed et al. (1998) who studied the effect of environmental pollutants on thirty four Egyptian buffaloes. They reported a significantly higher mean frequency of SCEs in the buffalo samples exposed to environmental toxicants (11.8±1.4) compared to the control group (8.3±1.2). Therefore, the results on SCE for Murrah and Nili-Ravi buffalo breeds obtained in the present study are closer to the control values obtained by Ahmed et al. (1998). In this study, a low frequency of SCEs and CAs was observed for Nili-Ravi and Murrah breeds compared to previous studies in Bhadawari, Surti and Toda buffaloes breeds (Murali et al., 1998; Murali et al., 2009; Vijh et al., 1995), thereby, suggesting that their genome has been strongly selected and more stable in comparison with other breeds of the same species.

In another study by Ahmed et al. (2001), a baseline frequency of 8.3±1.1 and 7.76±0.8 for SCE in Beheri and Saidi breed of Egyptian water buffaloes has been studied. The results obtained here are well below this baseline value suggesting the more stable genome integrity in Murrah and Nili-Ravi breeds. Our results are also confirmed by the study of Pires et al. (1998) who studied the CAs in one hundred and eighty two Murrah, Mediterranean and Jaffrabadi buffalo breeds reared in the state of Sao Paulo, Brazil. They observed a mean value of 3.01, 2.49 and 3.89 % CAs in Murrah, Mediterranean and Jaffrabadi breeds of buffalo respectively.

Therefore, the results obtained in this study indicate the better adaptive capacity of Nili-Ravi and Murrah buffalo breeds to withstand tropical climatic conditions. However, the SCE and CA level differed significantly between Murrah and Nili-Ravi, with Murrah breed showing the significantly higher SCE/cell and CAs compared to Nili-Ravi. Therefore, it can be concluded that
this better capacity in Nili-Ravi as revealed by the lower mean frequency of CAs and SCEs has been developed through long-term natural selection and evolution over the centuries. The naturally evolved breeds of Murrah and Nili-Ravi 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 Murrah and Nili-Ravi breeds of riverine buffalo, this information can serve as a baseline for future investigations. The observations made in the present study and the existing literature on other breeds of buffalo suggests that the CAs and SCEs in the chromosomes of riverine buffaloes have a wide range contributing to their superior adaptability and productivity traits that can be exploited in this scenario of climate change.

The variation in temperature and humidity conditions may not be the only significant differences observed to influence the genome integrity between the zebu cattle and buffalo breeds of arid and semiarid regions, but other abiotic factors and management practices could attribute to the variations observed in genome integrity. 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.

In the present study, the expression pattern of heat shock protein genes (HSP70.1, 70.2, 70.8, 40, 10 and HSF-1) in the lymphocytes of cattle (Sahiwal and Tharparkar) and buffalo (Murrah) during different season (summer, winter and thermoneutral) was carried out. Results indicated the significantly higher ($P \leq 0.001$) expression pattern of all the above mentioned heat shock protein genes during summer season and winter seasons compared to the thermoneutral. However, the magnitude of increase was remarkably higher ($P \leq 0.001$) during summer season compared to winter and thermoneutral. The results of the present study indicated that the expression pattern of HSP genes varied widely under natural environmental conditions.

The rapid induction of HSP70 and other small HSPs represents a unique feature of the physiological function of these molecules. It is well known that HSP expression is accomplished by mechanisms of transcriptional activation.
and translation involving heat shock transcription factors (HSFs). The ability to survive and adapt to heat stress appears to be a physiological process, as cellular stress responses are ubiquitous among both eukaryotes and prokaryotes and HSPs are involved in these responses (Lindquist, 1986). In particular HSP70 family represents the most highly inducible HSPs which are vitally important for thermo-tolerance. Our results are in accordance with results obtained by Banerjee et al. (2014) who reported that HSP70 genes were expressed during both summer and winter in goat breeds but the expression of HSP70.1, HSP70.6 and HSP70.8 was significantly higher during summer month. Kamwanja et al. (1994) have reported a 2 to 3 fold increase in the intracellular concentration of HSP70 after 1h exposure at 42°C in the lymphocytes of different cattle breeds. Lacetera et al. (2006) have also reported an increased HSP70.1 mRNA levels in PBMC of cattle. King et al. (2002) have reported an induction of HSP70 at 41°C in the lymphocytes of mice and concluded that HSP70 performed a significant role to help the survival of mice during high temperature conditions. The increase in the expression pattern of HSP genes in Murrah buffalo during summer season are in accordance with the previous study by Patir and Upadhyay, (2010) who reported a rise in HSP70 protein concentration after 2 h of exposure at 45°C in the PBMC of Murrah buffalo. Mishra et al. (2011) have also reported the 2.5 fold increase in HSP70 concentration in bovine lymphocytes cultured in vitro at higher temperature conditions.

The present study reveals that HSP70 gene induction varied among different cattle and buffalo breeds correlating with the normal range of environmental exposure. Cao et al. (2009) reported that higher heat stress level led to higher concentration of HSP70 in the testis and epididymis of mice. HSP70 response during different seasons could be attributed to breed specific differences. Interestingly, increased expression of HSP70 during hypothermic stress has been observed in humans, and this increased expression of heat shock proteins was possibly a natural protective and homeostatic response of the cell to the deleterious effects of the hypothermic stress (Rada et al., 2010). Several studies in bovine, murine, ovine and human cells give evidence that
constitutive elevation of HSPs in gene and protein levels provide protection upon heat stress (Collier et al., 2008). The results of present results can also be extended to the previous studies where heat stress-induced HSP70 expression was observed in the bovine lymphocytes (Liu et al., 2010; Guerriero and Raynes, 1990; Dangi et al., 2012), in kidneys of goats (Zulkifli et al., 2010), in myocardium (Gray et al., 2000), in lung cells (Fargnoli et al., 1990), and in hepatocytes and liver (Heydari et al., 1995; Hall et al., 2000) thereby indicating that heat shock proteins provide protection from toxic effects of thermal stress.

Mitochondrial HSPs may also play a role in the maintenance of metabolic activity and survival (Ohashi et al., 1995) and this may be a reason for higher level of HSP60 and HSP10 observed in the granulosa cell layer of healthy medium follicles. Stressful conditions are known to increase the misfolded proteins in the mitochondrial matrix and hence the levels of HSP10 along with its co-chaperonin HSP60. Lin et al. (2001) reported the higher expression of HSP60/HSP10 in cardiac myocytes and therefore concluded that these chaperonins may be particularly protective against apoptosis against simulated ischemia-reoxygenation. Furthermore, in earlier investigations, up-regulation of HSP60 expression in rat myocytes (Richard et al., 1995) and in human umbilical venous endothelial cells (Pfister et al., 2005) was observed during heat stress. Increased HSP60 mRNA expression during summer season in goats could evoke its transcription in PBMCs to prevent cell from damaging effect of heat stress like denaturation of proteins, and HSP60 helps in refolding of proteins and prevents aggregation of denatured proteins.

In the present study, it was observed that, during summer season, HSP90 mRNA expression was significantly ($P<0.05$) higher in Tharparkar, Sahiwal and Murrah buffalo in comparison to winter season. HSP90 mRNA expression was statistically non-significant ($P>0.05$) between the breeds during winter and summer seasons. Previous studies also reported increased HSP90 expression due to heat stress in human blood lymphocytes (Schimidt and Abdulla, 1988), T-cells (Ciavarra and Simeone, 1990), rat myocytes (Richard et al., 1995), heart, liver and kidney of broilers (Lei et al., 2009), murine embryonic fibroblast cells (Beckham et al., 2010), lung, heart, spleen, liver, and brain
(Sareh et al., 2011). Increased HSP90 expression during summer season in comparison to winter season in our study could be due to the fact that heat stress stimulated and quickly initiated the transcription of HSP90 mRNA and translation of HSP90 protein to protect cells from heat stress. The present findings, therefore, provide the clear evidence of induction in HSP70 super family (HSP70.1, 70.2 and 70.8) and other HSP genes (HSP90, 40, 10 and HSF-1) in the lymphocytes of cattle and buffalo during summer season compared to the winter and thermoneutral season. Collectively, these findings along with previous results in bovine lymphocytes provide compelling evidence for a major effect of high ambient temperature exposure during meiotic maturation on subsequent blastocyst development.

It is well established that environmental stressors have the potential to activate the hypothalamo-pituitary-adrenal cortical axis and sympatho-adrenal medullary axis (Minton, 1994). The secretion of cortisol stimulates physiological adjustments that enable an animal to tolerate the caused by a hot environment. In the current investigation, the level of cortisol was significantly higher ($P \leq 0.05$) in the Sahiwal and Tharparkar cattle of semi-arid (Karnal) zone as compared to that observed in the corresponding cattle breeds of arid zone (Bikaner). Also, the cortisol level differed significantly ($P \leq 0.01$) amongst the buffalo breeds Murrah and Nili-Ravi of the semiarid zone with the significantly higher ($P \leq 0.01$) level in Murrah buffalo. The lower cortisol level in zebu cattle breeds of arid zone (Sahiwal, Tharparkar and Kankrej) compared to their semiarid counterparts suggests that these indigenous breeds of cattle have evolved over the centuries through natural selection for adaptation to harsh climatic conditions. Contrary to the previous reports and perception, current findings indicate that the level of cortisol was significantly lower during increased THI conditions. Therefore, it may be substantiated that these indigenous breeds of cattle have adapted to the prevailing environmental condition of the native tract acquiring specific gene combination and further compromise their physiological processes in adjusting to the adapted habitat.
Contrary to the results obtained in the current investigation, increase in plasma cortisol concentration with the increase in ambient temperature in farm animals has been reported by several workers (Dhami et al., 2006; Yousef, 1985; Habeeb et al., 2001). Marked elevations of plasma glucocorticoids have been observed in cattle acutely exposed to high environmental temperatures (Stott and Wiersma, 1971). The increased cortisol levels in these cows could be regarded as a direct effect of heat stress from solar radiation, since stress conditions activate the hypothalamic-pituitary-adrenal axis. Wise et al. (1988) also reported higher serum cortisol levels in heat stressed cows as compared to the cows maintained under cool conditions. A significant increase in plasma concentration has been reported in heat stressed cows. Maria and Habeeb, (2010) also reported an increase in cortisol concentration with increase in ambient temperature. However in support of the present study, several workers have reported a significant decrease in the level of cortisol on exposure to higher ambient temperature. Collier et al. (1982) reported the decrease in plasma cortisol level in cows on exposure to chronic heat stress conditions. This discrepancy in results may be related to different responses to acute and chronic high ambient temperature resulting in altered adrenal metabolism. Christison and Johnson (1972) also indicated that, with chronic heat stress (high THI), plasma cortisol levels were significantly depressed as compared to values at thermoneutrality (18°C). Stott and Wiersma (1971) noted a similar trend in cortisol level in cattle exposed to a hot summer. Furthermore, the difference in the cortisol level may be related to the intensity and duration of exposure to high ambient temperature. Abilay et al. (1975) reported that a prolonged heat exposure (33.5°C) resulted in a general reduction effect of plasma cortisol concentration.

In this study, mean ± SD of plasma T₃ and T₄ concentration was significantly lower (P ≤ 0.01) in the zebu cattle breeds of arid zone compared to their semiarid counterparts. Our results are supported by the findings of Thompson (1973) who concluded that adaptation to high environmental
temperature caused an increase in body temperature and a consequent decrease in thyroid activity. There exists seasonal variation in thyroid gland activity, which in turn is related to ambient temperature and humidity (Khurana, 1983). In line with the present study, a decline in the plasma concentration of T₃ was observed in thermal exposed lactating cows by Johnston and Kucey, (1988). Acute elevation in THI has been reported to induce a decrease in plasma T₃ concentration in heifer and old buffalo calves (Nessim, 2004) and Friesian calves (Yousef et al., 1997). Magdub et al. (1982) and Pratt and Wetteman (1986) reported decreased T₃ and T₄ concentration in heat-stressed cows. This decreased concentration of T₃ in the cattle breeds of arid zone may be an adaptive response and also might be an attempt to reduce metabolic rate and heat production (West et al., 1999). Hassan and El-Nouty (1985) reported higher magnitude of reduction in thyroid activity in summer heat in buffalo than cows and attributed to difference in adaptive capacity to adapt to tropical conditions. Rasooli et al. (2004) reported significantly lower levels of T₃ and T₄ in Holstein heifers during hot summer compared to the winter.

There is increase in plasma concentration of cortisol and corticosterone and less frequently an increase in plasma epinephrine and nor epinephrine concentration in heat stressed animals (Minton, 1994). Magdub et al. (1982) reported that during heat stress there were significant reduction in concentrations of triiodothyronine (T₃) and thyroxine (T₄) in plasma and in milk of lactating cows. However, a significant increase in T₃ but not in T₄ level was observed during heat stress in crossbred cattle (Singh et al., 1984; Collier et al., 1982).

Many studied on physiological adaptation mechanisms such as rectal temperature, pulse rate and respiration rate in small ruminants (Sevi et al., 2001; Srikandakumar et al., 2003; Maurya et al., 2004; Marai et al., 2007; Otoikhian et al., 2009; Phulia et al., 2010; Sharma et al., 2013).Pulsation and respiration rate per minute was found to be increased by the effect of environmental temperature.
Physiological parameters like respiration rate, heart rate, body temperature and skin temperature gives an immediate response to the climatic stress and consequently the level of discomfort/comfort to the animal (Bianca, 1965). These responses have been used as a measure of dairy cow comfort and adaptability to an adverse environment or as a sensitive physiological measure of environmental modification (Roman-Ponce et al., 1977). Physiological responses like rectal temperature, pulse rate and respiration rate reflect the degree of stress imposed on animals by climatic parameters. The ability of an animal to withstand the rigors of climatic stress under warm conditions has been assessed physiologically by means of changes in body temperature, respiration rate and pulse rate (Leagates et al., 1991; Sethi et al., 1994).

Rectal temperature is generally considered to be a useful measure of body temperature and changes in RT indicates changes of a similar magnitude in deep body temperature (Herz and Steinhauf, 1978; Rosenberg et al., 1983). Change in rectal temperature has been considered an indicator of heat storage in animal’s body and may be used to assess the adversity of thermal environment, which can affect growth, lactation and reproduction of dairy animals (West et al., 1999). The rectal temperature is recognized as an important measure of physiological status as well as ideal indicator for assessment of stress in animals (Lefcourt et al., 1986). In the present study, no significant difference in the rectal temperature of cattle breeds was observed between arid and semiarid zone. The results obtained in this study indicate that the zebu cattle breeds (Sahiwal, Tharparkar and Kankrej) maintain homeothermy to the maximum possible level and therefore, are well adapted to the tropical conditions of India. Previous researchers have reported the elevation of rectal temperature during heat stress conditions. Although, present study obtained no significant difference in rectal temperature of Murrah and Nili-Ravi buffalo breeds, however, the RT of Nili-Ravi Buffalo breed was numerically lower than that of Murrah buffalo breed. Our study is further
Results & Discussion

supported by the findings of Mullick (1960) who reported that the rectal temperature during summer months under high and low humid conditions was less for buffaloes than cattle. Joshi and Tripathy (1991) noticed an increase in rectal temperature from 102.0°F to 103.8 °F when buffalo calves were exposed to 40.5°C for 8 hours daily for three months. It was recorded as 2.6°C rise in rectal temperature in buffaloes when exposed to direct sun rays in the months of June and July. Mullick (1964) reported that rectal temperature was higher during summer months in comparison to winter in Indian buffaloes. The rectal temperature was higher during summer (39.83°C) than autumn (38.30°C) in lactating cows (Padilla et al., 2006; Taneja, 1960). This study suggests that high temperature and relative humidity did not significantly affect the effectiveness of the evaporative cooling and the homeothermic status of the animals was being effectively countered by physical and physiological process of thermolysis (Joshi and Tripathy, 1991).

Respiratory rhythm has been reported to be the more sensitive index under tropical conditions for assessing the animal response to environmental changes and evaluating heat tolerance (Williams et al., 1960). Respiration rate is the most consistent of all the physiological responses studied and affected more by solar radiation than by other influences (Gaalas, 1945; Seath and Miller, 1946).

In the current investigation, significantly higher ($P \leq 0.05$) frequency of respiration was observed in the zebu cattle breeds (Sahiwal, Tharparkar and Kankrej) of semiarid zone as compared to their arid zone counterparts. Respiration rate is the first reaction when animals were exposed to environmental temperature above the thermo neutral zone. Increase in respiratory frequency may be used an index of discomfort in large animals. Also, the RR was significantly higher ($P \leq 0.05$) in Murrah buffalo in comparison to Nili-Ravi buffalo breed. This higher RR in cattle and buffalo breeds of semiarid zone suggests that in keeping the body temperature constant as indicated by the rectal temperature, respiration rate is increased to restore
normal heat balance [60]. Relatively less respiration rate in the zebu cattle breeds of arid zone indicates their higher magnitude of resilience in adapting to the harsh climatic conditions in this zone. Contrary to the results obtained in this study, previous researchers reported a very high positive correlation between the respiration rate and ambient temperature and it even raised up to 0.833 °C when humidity was constant in buffaloes (Bond and McDowell, 1972; Misra et al., 1963; Findlay and Ingram, 1961). An evaporative heat loss from the respiratory tract is regarded as one of the primary mechanisms for maintenance of heat balance (McDowell, 1976). This respiratory response arises from direct heat stimulation of peripheral receptors which transmit nervous impulses to the thermal centre in the hypothalamus. The cardio-respiratory centre is then stimulated to send impulses to the diaphragm and intercostal muscles for further respiratory activity (Razdan et al., 1969). A high RR in most cases did not necessarily indicate that the animal is successful in keeping its body temperature constant, but rather indicated that it is already overheated and trying to restore normal heat balance (McDowell, 1976). Chikamune and Shimizu (1983) observed a highly significant correlation between RR and seasonal air temperature in swamp buffalo and Holstein cows. Respiration rate increased from 29 to 59/minute when male buffalo calves were exposed to 40.5°C (Joshi and Tripathy, 1991). The increase in respiratory frequency was reported to be two and half times higher in heat stressed animals than control animals (Joshi and Tripathy, 1991). Das et al. (1999) observed an increase in respiration rate from 14 to 70/minute in the month of June in Murrah buffalo calves when exposed to direct sunlight for 6 hours. McLean (1963) reported that increase in respiration rate under high temperature and humidity conditions enabled the animal to dissipate the excess body heat by vaporizing more moisture in the expired air, and accounted for about 30 percent of the total heat dissipation. Salem (1980) reported an increase in respiration rate of buffaloes and crossbreds cattle during summer compared to other seasons. A higher respiration rate of
71.5/minute during summer compared to 38.8/minute in winter was recorded in lactating cows by Taneja (1960). Bianca and Findlay (1962) found a highly significant increase in the ventilation rate of cattle and buffaloes with increase in the ambient temperature. Bhatnagar and Choudhary (1960) reported that the combination of relative humidity and air temperature caused variation in body temperature and respiration rate of animals, whereas the relative humidity caused variation in pulse rate. In this study, pulse rate followed the same trend.