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# Association of SSCP Polymorphisms of *HSP70* Gene with Physiological, Production and Reproduction Performance in Sahiwal and Crossbred Cows

J. Sai Prasanna[1], S.T. Viroji Rao[1], M. Gnana Prakash[1],
Suresh Rathod[2], P. Kalyani[3], B. Rajith Reddy[1]

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## ABSTRACT

**Background:** Cellular tolerance to heat stress is mediated by heat shock proteins (*HSP*s). The *HSPs* act as molecular chaperones and are transcribed in response to stress. Among different families of these proteins, *HSP70* is considered to be related to the development of temperature tolerance. Unraveling polymorphism in heat shock protein genes could be a step towards the identification of genetic markers for selecting heat-tolerant cattle.

**Methods:** The present study was carried out in Sahiwal (n=50) and Crossbred cows (n=50) with the objective to identify polymorphisms in *HSP70* gene. Two fragments (295 and 220 bp) of *HSP70* gene were subjected to Polymerase Chain Reaction-Single-Strand Conformation Polymorphism (PCR-SSCP) technique. Statistical analysis was performed to study the association of each SSCP genotype on physiological, production and reproduction traits in Sahiwal and crossbred cows using the univariate GLM model of SPSS 25.

**Result:** The PCR-SSCP of 295 bp fragment of *HSP70* gene revealed two genotypes AA and AB in Sahiwal cows and two genotypes AA and AC in crossbred cows. The association analysis revealed that genotype AA had higher peak milk yield in Sahiwal cows while the same genotype had higher total lactation milk yield, lower service period and calving interval in crossbred cows. The 220 bp fragment was found to be monomorphic in both Sahiwal and crossbred cows.

**Key words:** Crossbred cows, Heat stress, *HSP70,* Polymorphisms, Sahiwal.

## INTRODUCTION

Ever increasing human population is exerting tremendous pressure on natural resources including livestock (Herrero and Thornton, 2013). Changing global environment is also posing a major threat to the sustainability of livestock production systems (Naqvi and Sejian, 2011; Polsky and Keyserlingk, 2017 and Rojas-Downing *et al.,* 2017). Global warming impairs production and reproduction performance, metabolic health and immune competence of livestock.

Selection and breeding methods are the only tools available for animal breeders to meet these challenges. Though crossbreeding was proved to be the fastest way of increasing milk production, adaptability of crossbreds was poor as reflected by lower breeding efficiency. Further, crossbreds are found to be highly susceptible to diseases and less tolerant to heat stress (Singh, 2016). Current strategies focussing on managemental manipulations to mitigate the effects of heat stress are partially successful and are only short term measures (Berman, 2005). Feed and housing modifications with focus on genetic manipulations could be a sustainable long term strategy to reduce the effects of heat stress (Boonkum *et al.,* 2011; Scholtz *et al.*, 2013).

Heat shock proteins (*HSP*s) are molecular chaperones that play a critical role in recovering cells from stress and cytoprotection, protecting the cells from subsequent insults. Through their ability to recognize nascent polypeptides, unstructured protein areas and exposed hydrophobic stretches of amino acids, they protect stressed cells. Chaperones thus hold, translocate or refold denatured proteins and prevent their irreversible aggregation with other cell proteins (Archana *et al.*, 2017).

Genes encoding the heat shock proteins are called *HSP* genes and their nomenclature is given by the HUGO Gene Nomenclature Committee. Though there are many *HSP* genes, thermo tolerance is mainly correlated with *HSP70* and *HSP90* genes in Livestock species. *HSP70* is reported to be the most abundant and temperature-sensitive playing a crucial role in environmental stress and thermal adaptation

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[1]Department of Animal Genetics and Breeding, College of Veterinary Science, Rajendranagar, Hyderabad-500 030, Telangana, India.
[2]Department of Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad-500 030, Telangana, India.
[3]Department of Veterinary Biotechnology, College of Veterinary Science, Rajendranagar, Hyderabad-500 030, Telangana, India.

**Corresponding Author:** J. Sai Prasanna, Department of Animal Genetics and Breeding, College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad-500 030, Telangana, India. Email: drsaiprasannareddy@gmail.com

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(Gade *et al*., 2010). In farm animals elevated levels of expression of proteins of the *HSP70* and *HSP90* family were observed in sheep, buffalo, cattle, broilers and goats during the summer season (Archana *et al.,* 2017). Polymorphism in the *HSP70* and *HSP90* genes have shown an association with heat tolerance, milk production, fertility and disease susceptibility in livestock (Shergojry *et al*., 2014; Kumar *et al*., 2015, Bhat *et al.,* 2016). They can make ideal candidate gene markers for the selection of animals with better climate resilience, immune response and superior performance. (Hassan *et al.,* 2019).

Although differences in thermo tolerance at physiological and cellular level are documented (Collier *et al.,* 2006; Chaiyabutr *et al.,* 2008; Wilson and Crandall, 2010 and Dalcin *et al.,* 2016) in both *Bos indicus* and *Bos taurus* cattle, information on polymorphism of *HSP* genes in Sahiwal cattle and Holstein Friesian crossbreds is scarce. There are few reports from India regarding the association of *HSP70* and *HSP90* gene polymorphism with heat tolerance in Tharparkar cattle (Bhat *et al.,* 2016), Deoni cattle (Kerekoppa *et al*., 2015), Jersey crossbred cows (Sailo *et al.,* 2015) and from abroad in Holstein cow (Li *et al.,* 2011). Therefore in the present study, polymorphisms of HSP70 and their correlation with various physiological, production and reproduction traits in Sahiwal and crossbred cows were explored.

## MATERIALS AND METHODS

### Experimental animals

A total of 50 Sahiwal cows maintained at the Livestock Farm Complex, College of Veterinary Science Rajendranagar and 50 crossbred cows (Holstein Friesian × Sahiwal crosses with 7/8 exotic inheritance maintained at the Military Dairy Farm, Secunderabad were utilized for the present investigation.

### Weather conditions

Hyderabad, the capital city of Telangana State is located at 17.366°N Latitude and 78.476°E Longitude. It is situated at an elevation of 536 meters (1607 feet) above the mean sea level. The data on the weather conditions, maximum and minimum temperatures (°C), dry and wet bulb readings (°C) and relative humidity (%) during the experimental period were collected from Agriculture Climate Research Center, ARI, Hyderabad. During the present study year of 2018, the average environmental temperatures ranged from 27.92°C in December which was the coldest month to 41.24°C in May, which was the hottest month. Hyderabad experienced moderate rainfall in July, August and September. The average annual rainfall received was 766 mm and the average relative humidity was around 65% varying from 45% during summer to 78% during the monsoon. Winters were moderately cold with temperatures ranging from 15°C to 31°C.

### Genomic DNA isolation

About 10 ml of peripheral blood was collected aseptically from each of the representative cows from the external jugular vein into a sterile vacutainer tube containing 0.5 percent EDTA. Genomic DNA of each of the animals was isolated from the blood samples using the standard phenol-chloroform extraction method as described by Green and Sambrook (2012), with minor modifications. The purity of genomic DNA samples was assessed by measuring the optical densities (OD) at 260 nm and 280 nm against blank using Nanodrop (Thermo Fisher Scientific). The samples which gave the OD ratio between 1.7 and 1.9 were assessed as good and used for PCR amplification.

### Physiological parameters

Data about the physiological parameters, respiration rate (RR) and rectal temperature (RT) of each animal under the present study were recorded twice daily for 30 days in each of the three seasons *i.e* during May (2018) for summer, August (2018) for rainy and from mid-December (2018) to mid-January (2019) for winter season respectively and the average was taken as final reading for each cow in association analysis. The timings of recording the physiological parameters were 8 AM and 2 PM. The Heat Tolerance Coefficient (HTC) based on respiration rate and rectal temperature was calculated for each animal using the formula given by Benezra (1954).

### Production and reproduction traits

Data on each animal about different aspects like animal no., sire no., dam no., date of birth, date of calving, lactation length and lactation milk yield, *etc*., were collected from the history sheet/daily farm registers maintained at the concerned farms. The various production and reproduction traits like total lactation milk yield (TLMY), peak yield (PY), lactation length (LL), service period (SP), dry period (DP) and calving interval (CI) were calculated from the available data in both Sahiwal and crossbred cows.

### PCR primers and amplifications

Two pairs of primers (procured from BioServe Biotechnologies Pvt Ltd, Hyderabad) specific for the desired regions (295 and 220 bp) of the initial coding region of *HSP70* gene as available in the literature (Bhat *et al.,* 2016) were used to amplify the targeted regions. The details of primer sequences, length of the primer (bp), melting temperature (Tm) are presented below.

| Gene fragment | | Sequence (5′-3′) | Length of the primer (bp) | Meltingt temperature (Tm,°C) |
|---|---|---|---|---|
| Fragment I 295 bp | F | AAACATGGCTATCGGCATCGACCT | 24 | 57 |
| | R | AGGCTTGTCTCCGTCGTTGATGA | 23 | 57 |
| Fragment II 220 bp | F | CTAAGGTGCAGGTGAGCTACAAAG | 24 | 57 |
| | R | TTGATGATCCTCAGCACGTTCAGC | 24 | 57 |

F = Forward; R = Reverse.

The PCR reactions were carried out on a total of 12.5 µl volume containing template DNA of 1 µl (50-100 ng/µl), 1.0 µl each of forward and reverse primers (10pM), 2.5 µl of 10X Taq buffer, 0.8 µl of dNTPs (10 mM), 0.125 µl of Taq Polymerase (5 units/µl) and 6.075 µl of Nuclease free water. Amplification was done in a pre-programmed thermo cycler (Prima-Duo, Himedia labs). The PCR cycling conditions involved an initial denaturation at 95°C for 5 min, followed by 35 cycles with initial denaturation at 95°C for 30 sec, annealing temperature of 50°C for 35 sec, extension at 72°C for 30 sec followed by a final extension at 72°C for 10 min. PCR products were detected by electrophoresis on 2% agarose gel stained with ethidium bromide.

### Single strand conformation polymorphism (SSCP)

The presence of variation in the fragments of *HSP70* gene was screened using the single-strand conformation polymorphism (SSCP) technique using the amplified PCR products. Polymorphism was identified basing on the band pattern observed in the SSCP gels after silver staining (Bassam *et al*., 2007). The different patterns observed were recorded for further analysis. The most common band pattern identified was named as A. If there are more bands, in addition to the common bands, they were marked as B, C, *etc*., depending on the band pattern.

### Genotype and allele frequencies

Genotype frequencies for variant genotypes were calculated using the formula:

$$\text{Genotype frequency} = \frac{\text{No. of animals with specific genotype } (AA,\ AB \text{ or } BB)}{\text{Total no. of animals}}$$

Allele frequencies were calculated as follows:

$$\text{Allele frequency of } A = AA + 1/2\ AB$$

$$\text{Allele frequency of } B = BB + 1/2\ AB$$

Where,
*AA* and *BB* = Genotype frequencies of homozygotes.
*AB* = Genotype frequency of heterozygote.
*A* and *B* = Allele frequencies.

### Association analysis

Statistical analysis was performed to study the association of each SSCP genotype on physiological, production and reproduction traits in Sahiwal and Crossbred cows. Data on physiological traits was corrected for season effect and used for association analysis. The univariate GLM model of SPSS 25 was used to perform the analysis according to the following statistical model:

$$Y_{ijk} = \mu + G_i + P_j + e_{ijk}$$

Where,
$Y_{ijk}$ = Dependent variable (respiration rate, rectal temperature, heat tolerance coefficient, total lactation milk yield, peak yield, lactation length, gestation period, service period, dry period and calving interval).
$\mu$ = Overall mean.
$G_i$ = Effect of i$^{th}$ SSCP genotype (i= 1…n).
$P_j$ = Effect of j$^{th}$ parity of the animal at the time of blood collection (j= 1…n).
$e_{ijk}$ = Random error assumed to be distributed normally and independently with mean zero and variance $\sigma^2_e$.

Significant differences between the means of different genotypes and parities were tested by Duncan's multiple range test (DMRT). Values were considered significant at P≤0.05 and presented as means±standard errors.

## RESULTS AND DISCUSSION

The representative image showing the PCR amplified products of Fragment I and Fragment II of *HSP70* gene, showing the sizes of 295 bp and 220 bp are presented in Fig 1 and 2 respectively, while the PCR-SSCP polyacrylamide gel images are presented in Fig 3 and 4 respectively. The Fragment I (295 bp) of the *HSP70* gene was found to be polymorphic in both Sahiwal and crossbred cows. In Sahiwal, two SSCP genotypes namely *AA* and *AB* were documented whose frequencies were estimated to be 0.58 and 0.42, respectively while in crossbred cows, two SSCP patterns *AA* and *AC* were documented with frequencies 0.62 and 0.38, respectively (Fig 3).

The Fragment II (220 bp) fragment of *HSP70* gene was found to be monomorphic in both the breeds studied

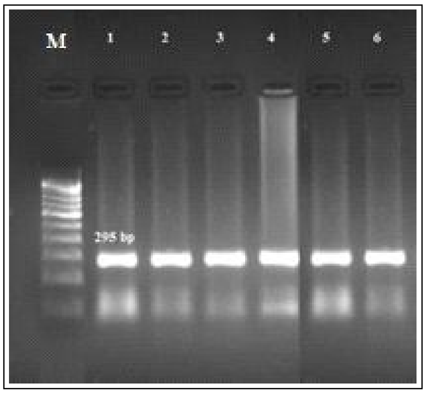

**Fig 1:** Agarose gel electrophoresis image showing PCR amplified product (295 bp) of *HSP70* gene.

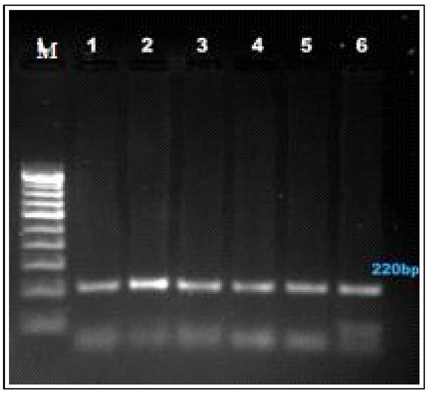

**Fig 2:** Agarose gel electrophoresis image showing PCR amplified product (220 bp) of *HSP70* gene.

(Fig 4) and hence, was not considered for further association analysis.

The means obtained from the statistical analysis for the effect of *HSP70* fragment I genotypes on the physiological, production and reproduction traits in Sahiwal and crossbred cows are presented in Table 1, 2 and 3 respectively.

### Association analysis of *HSP70* gene polymorphism with physiological traits

The SSCP genotypes of Fragment I of *HSP70* gene had no significant effect on physiological parameters studied in both Sahiwal and crossbred cows. However, Bhat *et al.* (2016) found allele *A* of *HSP70* to have positive correlation with thermal tolerance and genotype *AA* demonstrated superior heat tolerance. Various other authors also suggested that polymorphism in the *HSP70* gene could be used in genetic improvement programmes of cattle for heat tolerance (Cai *et al.*, 2005; Li *et al.*, 2011; Deb *et al.*, 2013 and Sodhi *et al.,* 2013).

### Association with production traits

The SSCP genotype *AA* of Fragment I of *HSP70* gene had significantly higher peak yield of 10.77 kg in Sahiwal cows and higher total lactation milk yield (3005.01 kg) in crossbred cows. The present investigation established a relationship between the *HSP70* Fragment I genotypes and production traits, with allele *A* having a positive effect on milk yield.

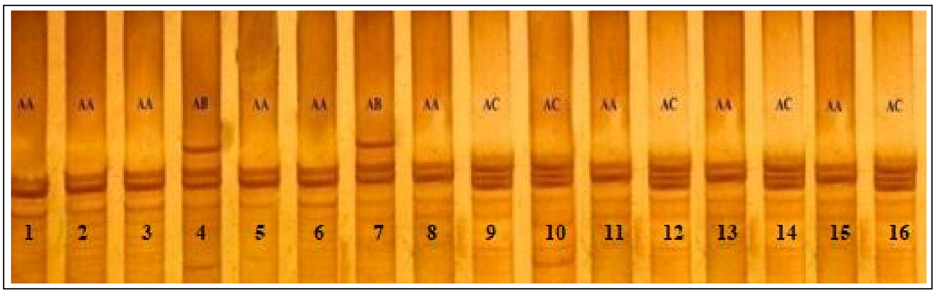

**Fig 3:** Polyacrylamide gel electrophoresis showing PCR-SSCP patterns for 295 bp fragment of *HSP70* gene. Lane 1-8 Sahiwal; Lane 9-16 Crossbred cows.

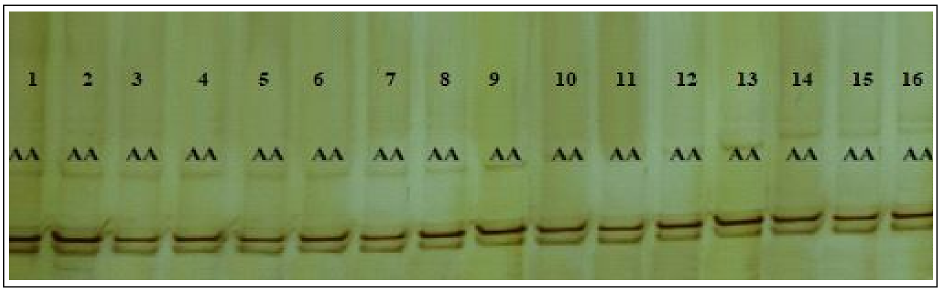

**Fig 4:** Polyacrylamide gel electrophoresis showing PCR-SSCP patterns for 220 bp fragment of *HSP70* gene. Lane 1-8 Sahiwal; Lane 9-16 Crossbred cows.

**Table 1:** Means of *HSP70* Fragment I genotypes and parity effects for production traits in Sahiwal and Crossbred cows.

| Effect | Sahiwal | | | | | | | Crossbreds | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | TLMY | | PY | | LL | | n | TLMY | | PY | | LL | |
| | | Mean | SE | Mean | SE | Mean | SE | | Mean | SE | Mean | SE | Mean | SE |
| | 50 | 1775.70 | 107.38 | 9.96 | 0.39 | 266.91 | 12.84 | 50 | 2836.10 | 95.14 | 13.58 | 0.48 | 328.28 | 2.64 |
| **Genotype** | | | | | | | | | | | | | | |
| *AA* | 29 | 1760.88 | 135.75 | $10.77^{a}$ | 0.49 | 291.17 | 291.17 | 31 | $3005.01^{a}$ | 115.50 | 14.39 | 0.59 | 332.97 | 3.20 |
| *AB* | 21 | 1790.51 | 162.69 | $9.15^{b}$ | 0.58 | 242.65 | 242.65 | | - | - | - | - | - | - |
| *AC* | - | - | - | - | - | - | - | 19 | $2667.19^{b}$ | 143.72 | 12.78 | 0.73 | 323.59 | 3.98 |
| **Parity** | | | | | | | | | | | | | | |
| 1 | 13 | 1964.88 | 198.72 | 10.62 | 0.71 | 317.79 | 23.77 | 5 | $2581.30^{c}$ | 314.81 | 13.27 | 1.60 | $336.23^{ab}$ | 8.72 |
| 2 | 11 | 1606.12 | 216.06 | 9.47 | 0.77 | 240.52 | 25.85 | 7 | $3399.18^{a}$ | 243.41 | 16.36 | 1.24 | $337.94^{a}$ | 6.74 |
| 3 | 18 | 1971.79 | 170.33 | 11.19 | 0.60 | 280.33 | 20.37 | 12 | $3239.35^{ab}$ | 179.80 | 14.12 | 0.91 | $321.28^{c}$ | 4.98 |
| 4 | 8 | 1560.01 | 258.45 | 8.56 | 0.92 | 228.99 | 30.92 | 9 | $3499.99^{a}$ | 205.56 | 16.97 | 1.04 | $322.81^{c}$ | 5.69 |
| 5 | - | - | - | - | - | - | - | 9 | $3306.44^{a}$ | 203.23 | 14.52 | 1.03 | $312.73^{d}$ | 5.63 |
| 6 | - | - | - | - | - | - | - | 8 | $3050.13^{b}$ | 205.56 | 14.26 | 1.04 | $330.96^{b}$ | 5.69 |

Means with similar superscripts in a column do not differ significantly ($P \leq 0.05$).

**Table 2:** Means of *HSP70* Fragment I genotypes and parity effects for production traits in Sahiwal and Crossbred cows.

| Effect | Sahiwal | | | | | | | Crossbreds | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | TLMY | | PY | | LL | | n | TLMY | | PY | | LL | |
| | | Mean | SE | Mean | SE | Mean | SE | | Mean | SE | Mean | SE | Mean | SE |
| | 50 | 1775.70 | 107.38 | 9.96 | 0.39 | 266.91 | 12.84 | 50 | 2836.10 | 95.14 | 13.58 | 0.48 | 328.28 | 2.64 |
| **Genotype** | | | | | | | | | | | | | | |
| *AA* | 29 | 1760.88 | 135.75 | 10.77$^{a}$ | 0.49 | 291.17 | 291.17 | 31 | 3005.01$^{a}$ | 115.50 | 14.39 | 0.59 | 332.97 | 3.20 |
| *AB* | 21 | 1790.51 | 162.69 | 9.15$^{b}$ | 0.58 | 242.65 | 242.65 | - | - | - | - | - | - | - |
| *AC* | - | - | - | - | - | - | - | 19 | 2667.19$^{b}$ | 143.72 | 12.78 | 0.73 | 323.59 | 3.98 |
| **Parity** | | | | | | | | | | | | | | |
| 1 | 13 | 1964.88 | 198.72 | 10.62 | 0.71 | 317.79 | 23.77 | 5 | 2581.30$^{c}$ | 314.81 | 13.27 | 1.60 | 336.23$^{ab}$ | 8.72 |
| 2 | 11 | 1606.12 | 216.06 | 9.47 | 0.77 | 240.52 | 25.85 | 7 | 3399.18$^{a}$ | 243.41 | 16.36 | 1.24 | 337.94$^{a}$ | 6.74 |
| 3 | 18 | 1971.79 | 170.33 | 11.19 | 0.60 | 280.33 | 20.37 | 12 | 3239.35$^{ab}$ | 179.80 | 14.12 | 0.91 | 321.28$^{c}$ | 4.98 |
| 4 | 8 | 1560.01 | 258.45 | 8.56 | 0.92 | 228.99 | 30.92 | 9 | 3499.99$^{a}$ | 205.56 | 16.97 | 1.04 | 322.81$^{c}$ | 5.69 |
| 5 | - | - | - | - | - | - | - | 9 | 3306.44$^{a}$ | 203.23 | 14.52 | 1.03 | 312.73$^{d}$ | 5.63 |
| 6 | - | - | - | - | - | - | - | 8 | 3050.13$^{b}$ | 205.56 | 14.26 | 1.04 | 330.96$^{b}$ | 5.69 |

Means with similar superscripts in a column do not differ significantly ($P \leq 0.05$).

**Table 3:** Means of *HSP70* Fragment I genotypes and parity effects for reproduction traits in Sahiwal and Crossbred cows.

| | Sahiwal | | | | | | | Crossbreds | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | SP (days) | | DP (days) | | CI (days) | | n | SP (days) | | DP (days) | | CI (days) | |
| | | Mean | SE | Mean | SE | Mean | SE | | Mean | SE | Mean | SE | Mean | SE |
| Overall | 44 | 149.85 | 8.16 | 140.56 | 7.13 | 438.31 | 6.79 | 46 | 202.29 | 9.20 | 157.03 | 7.39 | 418.56 | 7.73 |
| **Genotype** | | | | | | | | | | | | | | |
| *AA* | 25 | 161.74 | 10.40 | 139.48 | 9.09 | 425.89 | 8.66 | 30 | 183.29$^{b}$ | 11.05 | 151.48 | 8.88 | 404.14$^{b}$ | 9.28 |
| *AB* | 19 | 137.96 | 12.12 | 141.64 | 10.58 | 450.73 | 10.09 | 16 | 221.28$^{a}$ | 14.47 | 162.58 | 11.63 | 432.97$^{a}$ | 12.15 |
| *AC* | - | - | - | - | - | - | - | 30 | 183.29$^{b}$ | 11.05 | 151.48 | 8.88 | 404.14$^{b}$ | 9.28 |
| **Parity** | | | | | | | | | | | | | | |
| 1 | 13 | 192.99$^{a}$ | 14.05 | 144.15 | 12.28 | 463.12 | 11.70 | 5 | 185.00 | 29.76 | 158.15 | 23.93 | 442.53 | 25.00 |
| 2 | 11 | 145.10$^{b}$ | 15.28 | 168.64 | 13.35 | 421.13 | 12.72 | 7 | 247.80 | 23.01 | 136.49 | 18.50 | 428.12 | 19.33 |
| 3 | 14 | 136.60$^{b}$ | 13.71 | 127.09 | 11.98 | 448.19 | 11.42 | 11 | 212.77 | 18.48 | 174.87 | 14.85 | 418.10 | 15.52 |
| 4 | 6 | 124.70$^{b}$ | 20.83 | 122.36 | 18.19 | 420.81 | 17.34 | 7 | 185.33 | 20.94 | 162.83 | 16.84 | 421.17 | 17.59 |
| 5 | - | - | - | - | - | - | - | 8 | 192.42 | 20.42 | 170.54 | 16.42 | 421.87 | 17.16 |
| 6 | - | - | - | - | - | - | - | 8 | 186.83 | 20.94 | 150.83 | 16.84 | 416.50 | 17.59 |

Means with similar superscripts in a column do not differ significantly ($P \leq 0.05$).

It may be suggested that AA genotype may be incorporated in marker-assisted selection to increase milk yield in both Sahiwal and crossbred cows. Rosenkrans *et al.* (2010) also reported polymorphisms in the promoter region of the *HSP70* gene, some of which were associated with the milk yield in crossbred Brahman cows which may be useful in selecting cows with greater fertility.

### Association analysis with reproduction traits

The SSCP genotypes of Fragment I of *HSP70* gene had a non-significant effect on the reproductive traits studied in both Sahiwal and crossbred cows except on service period in crossbreds. Crossbred cows with AA genotype had a longer service period. Rosenkrans *et al.* (2010) also reported polymorphisms in the promoter region of the *HSP70* gene, some of which were associated with calving rates in crossbred Brahman cows which may be useful in selecting cows with greater fertility.

## CONCLUSION

The present study revealed that the different SSCP genotypes of the 295 bp fragment of *HSP70* gene were associated with production and reproduction traits and indicated their possible role in marker-assisted selection (MAS). However, the SSCP patterns obtained in the present study and their association analysis with physiological parameters did not reveal significant differences among the genotypes indicating that a larger population of cows with a wide genetic base may be needed to elucidate the association of polymorphism.

## REFERENCES

Archana, P.R., Aleena, J., Pragna, P., Vidya, M.K., Abdul Niyas, P.A., Bagath, M., Krishnan, G., Manimaran, A., Beena, V., Kurien, E.K., Sejian, V. and Bhatta, R. (2017). Role of heat shock proteins in livestock adaptation to heat stress. Journal of Dairy, Veterinary and Animal Research. 5(1): 13-19.

Bassam, B.J. and Gresshoff, P.M. (2007). Silver staining DNA in polyacrylamide gels. Nature Protocols. 2(11): 2649-54.

Benezra, M.V. (1954). A new index for measuring the adaptability of cattle to tropical conditions. Journal of Animal Science. 13: 1015-1015.

Berman, A.J. (2005). Estimates of heat stress relief needs for Holstein dairy cows. Journal of Animal Science. 836: 1377-1384.

Bhat, S., Kumar, P., Kashyap, N., Deshmukh, B., Dige, M.S., Bhushan, B., Chauhan, A., Kumar, A. and Singh, G. (2016). Effect of heat shock protein 70 polymorphism on thermotolerance in Tharparkar cattle. Veterinary World. 92: 113-117.

Boonkum, W., Misztal, I., Duangjinda, M., Pattarajinda, V., Tumwasorn, S. and Sanpote, J. (2011). Genetic effects of heat stress on milk yield of Thai Holstein crossbreds. Journal of Dairy Science. 94: 487-492.

Cai, Y., Liu, Q., Xing, G., Zhou, L., Yang, Y., Zhang, L., Li, J. and Wang, G. (2005). Polymorphism of the promoter region of *HSP70* gene and its relationship with the expression of *HSP70mRNA*, *HSF1mRNA, Bcl-2mrna* and *Bax-AMrna* in lymphocytes in peripheral blood of heat shocked dairy cows. Asian-Australasian Journal of Animal Sciences. 18(5): 734-740.

Chaiyabutr, N., Chanpongsang, S. and Suadsong, S. (2008). Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. International Journal of Biometerology. 52: 575-585.

Collier, R.J., Dahl, G.E. and Van Baale, M.J. (2006). Major advances with environmental effects on dairy cattle. Journal of Dairy Science. 89: 1244-1253.

Dalcin, V., Fischer, V., Daltro, D., Alfonzo, M., Priscila, E., Marcelo, S., Giovani, K., Marcos, S. and Concepta, M. (2016). Physiological parameters for thermal stress in dairy cattle. Revista Brasileira de Zootecnia. 45: 458-465.

Deb, R., Sajjanar, B. and Singh, U. (2013). Promoter variants at AP2 box region of Hsp701 affect thermal stress response and milk production traits in Frieswal cross bred cattle. Gene. 9: 37. DOI: 10.1016/j.gene.2013.09.037.

Gade, N., Mahapatra, R.K., Sonawane, A., Singh, V.K., Doreswamy, R. and Saini, M. (2010). Molecular characterization of *Heat Shock Protein 70-1* gene of goat (*Capra hircus*). Molecular Biology International. Article ID 108429.

Green, M.R., Sambrook, J. (2012). Molecular Cloning: A Laboratory Manual. New York, USA: Cold Spring Harbor.

Hassan, F., Nawaz, A., Rehman, M.S., Ali, M.A., Dilshad, S.M.R. and Yang, C. (2019). Prospects of *HSP70* as a genetic marker for thermo-tolerance and immuno-modulation in animals under climate change scenario. Animal Nutrition. 54: 340-50.

Herrero, M. and Thornton, P.K. (2013). Livestock and global change: Emerging issues for sustainable food systems. Proceedings of National Academy of Sciences of the United States of America. 110(52): 20878-20881.

Kerekoppa, R., Rao, A., Basavaraju, M., Geetha, G., Krishnamurthy, L., Narasimha, L., Das, D. and Mukund, K. (2015). Molecular characterization of the *HSPA1A* gene by single-strand conformation polymorphism and sequence analysis in Holstein-Friesian crossbred and Deoni cattle raised in India. Turkish Journal of Veterinary and Animal Sciences. 39: 128-133.

Kumar, R., Gupta, I.D., Verma, A., Verma, N., Nishant and Vineeth, M.R. (2015). Single nucleotide polymorphisms in *Heat Shock Protein* (*HSP*) *90AA1* gene and their association with heat tolerance traits in Sahiwal cows. Indian Journal of Animal Research. 51: 64-69.

Li, Q., Han, J., Du, F., Ju, Z., Huang, Z., Wang, J., Li, R., Wang, C. and Zhong, J. (2011). Novel SNPs in *HSP70A1A* gene and the association of polymorphisms with thermo tolerance traits and tissue specific expression in Chinese Holstein cattle. Molecular Biology Reports. 38: 2657-2663.

Naqvi, S. and Sejian, V. (2011). Global Climate Change: Role of Livestock. Asian Journal of Agricultural Sciences. 3(1): 19-25.

Polsky, L. and Keyserlingk, M.A.G. von. (2017). Invited review: Effects of heat stress on dairy cattle welfare. Journal of Dairy Science. 100(11): 8645-8657.

Rojas-Downing, M.M., Nejadhashemi, A.P., Harrigan, T. and Woznicki, S.A. (2017). Climate change and livestock: Impacts, adaptation and mitigation. Climate Risk Management. 16: 145-163.

Rosenkrans, C., Banks, A., Reiter, S. and Looper, M. (2010). Calving traits of crossbred Brahman cows are associated with *Heat Shock Protein 70* genetic polymorphisms. Animal Reproduction Science. 119(3-4): 178-182.

Sailo, L., Gupta, I.D., Verma, A., Chaudhari, M.V., Das, R., Upadhyay, R.C. and Goswami, J. (2015). Single nucleotide polymorphisms in *HSP90AB1* gene and its association with thermo tolerance in Jersey crossbred cows. Animal Science Reporter. 9: 43-49.

Scholtz, M., Maiwashe, A., Neser, F., Theunissen, A., Olivier, W., Mokolo-bate, M and Hendriks, J. (2013). Livestock breeding for sustainability to mitigate global warming, with the emphasis on developing countries. South African Journal of Animal Science. 43(3): 269-281.

Shergojry, S.A., Ramesha, K.P., Mir, N.A. and Aarif, O. (2014). Nucleotide sequence polymorphism within Exon 8 of Heat Shock Protein *HSP 90AA1* gene and its association with milk production traits in Deoni cows. Indian Journal of Animal Research. 48(5): 408-412.

Singh, C.V. (2016). Cross-breeding in cattle for milk production: Achievements, challenges and opportunities in India- A review. Advances in Dairy Research. 4(3): 158. DOI: 10.4172/2329-888X.1000158.

Sodhi, M., Mukesh, M., Kishore, A., Mishra, B.P., Kataria, R.S. and Joshi, B.K. (2013). Novel polymorphisms in UTR and coding region of inducible *Heat Shock Protein 70-1* gene in tropically adapted Indian zebu cattle (*Bos indicus*) and riverine buffalo (*Bubalus bubalis*). Gene. 527(2): 606-615.

Wilson, T. and Crandall, C. (2010). Effect of thermal stress on cardiac function. Exercise and Sport Sciences Reviews. 39(1): 12-17.