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Research Article

HSP90AB1 Variations in Four Cattle Breeds Raised in Türkiye

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Abstract: Cattle play an essential role in ensuring global food security, yet they are encountering growing challenges from climate change. In Türkiye, cattle rearing is a fundamental aspect of agriculture, and native breeds are particularly important, even as their numbers dwindle. Understanding the genetic makeup of these breeds, especially their heat tolerance, is of great importance for sustainable livestock production. This study focused on identifying polymorphisms in the *HSP90AB1* gene associated with heat tolerance in 122 cattle raised in Türkiye (Anatolian Black-AB, East Anatolian Red-EAR, Turkish Grey Steppe-TGS, and cosmopolitan Holstein Friesian-HF). The samples were respectively obtained from village herds in Antalya, Erzurum, Balıkesir, and Erzurum provinces for AB, TGS, EAR, and HF breeds. Blood samples were collected from different populations and genotyping was performed using Allele-Specific PCR. Regarding the *HSP90AB1* gene, polymorphism was observed in the populations, with two alleles (C and G) and three genotypes (CC, CT, and TT), while the CC allele was not observed in TGS cattle. The frequency of the T allele was higher than that of the C allele in the studied cattle population. The TT genotype frequency ranged from 0.12 (HF) to 0.38 (TGS), while the lowest (0.00) and highest (0.35) CC frequencies were observed in TGS and HF, respectively. Significant deviation ($p<0.05$) from Hardy-Weinberg Equilibrium (HWE) was detected in the EAR and TGS cattle breeds with respect to the *HSP90AB1* gene. As climate change intensifies environmental stresses, the adoption of molecular genetics and Marker-Assisted Selection (MAS) is necessary for the conservation of native genetic resources and the establishment of resilient livestock populations in Türkiye's changing climate environment.

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1. Introduction

Cattle, possessing adaptability to diverse global climatic regions, stand as the predominant animal species pivotal in the conversion of plant-derived resources and select industrial residuals, which

are not directly utilizable by humans, into high-quality animal protein suitable for human consumption (Demir et al., 2021; Şahin et al., 2023). Based on the data provided by the FAO in 2021, cattle contribute to roughly 91% of the worldwide milk output and approximately 35% of the global red meat yield (FAO, 2021). In Türkiye, the cattle population is recorded at 16 851 956, consisting of 49.2% cosmopolitan cattle breeds, 43.5% crossbreeds between native and commercial breeds, and 7.3% native breeds (TUIK, 2023). Over the past two decades, the scientific community has meticulously monitored and analyzed the impacts of climate fluctuations associated with global warming on a global scale. Since 1975, there has been a gradual rise in global temperatures, averaging 0.15-0.20 °C per decade, with projections suggesting a further increase of 2-3 °C by the year 2100 (Malhi et al., 2021). This anticipated climate change is expected to have significant consequences, including a decline in biological diversity and an increase in the prevalence of certain diseases and pathogens (Lewis Baida et al., 2021).

In light of the challenges posed by a growing global population and the depletion of natural resources, including diminishing water reserves, shrinking agricultural and grazing lands, and increasing environmental stresses such as climate change and disease outbreaks, it is imperative for humanity to make concerted efforts to ensure food security. Climate change, which has a significant global impact, is widely recognized as a major threat to the sustainability of livestock farming (Skuce et al., 2013; Rauw et al., 2020; Demir et al., 2021). The indigenous animal genetic resources and the genetic diversity within them are highly important in studies addressing adaptation to high temperatures or other extreme conditions in livestock. Geographic and climatic variations, as well as different breeding systems and purposes, have resulted in the formation of hundreds of breeds or subgroups over centuries, offering significant opportunities for environmental adaptation in terms of genotypic structure. Recently, there has been a worldwide shift towards favoring commercial livestock breeds over indigenous ones (Castillo et al., 2021). This trend has led to a notable decline in the population of indigenous livestock breeds. Many indigenous breeds have either been crossbred with high-yielding varieties or completely abandoned (Demir et al., 2021). However, there is a considerable likelihood that specific genes or gene combinations inherent in indigenous breeds will prove pivotal in commercial contexts. The number of native Turkish cattle populations is gradually decreasing, with this decline attributed to farmers' preference for commercial varieties due to the relatively low milk and meat yields of native breeds. Particularly, some native cattle breeds in Türkiye, such as Kultak, Dörtyol, Diyarbakır, and Alacadağ, are facing declining interest and gradual extinction due to their limited production capacities (Ertuğrul et al., 2015). Despite their lower yields, indigenous breeds exhibit greater resilience to diseases, suboptimal husbandry practices, and adverse environmental conditions.

In recent years, studies on tolerance to heat stress or high temperatures in farm animals have highlighted Heat Shock Proteins (HSP) and their associated gene regions (Demir et al., 2022; Suhendro et al., 2024). Numerous studies have emphasized the importance of the relationship between *HSP* genes and adaptation to heat stress in various farm animals such as cattle (Onasanya et al., 2021), sheep (Yurdagul et al. 2023), goats (Sahu et al., 2021), and chickens (Kim et al., 2022). HSP90, an exceptionally abundant chaperone protein found in all eukaryotic cells, experiences a marked increase in production following exposure to high temperatures (42-46 °C). The most important members of the HSP90 family are the isoforms HSP90 alpha (HSP90a) and HSP90 beta (HSP90b), expressed by two different genes (Sarman et al., 2021). While numerous studies have been conducted on *HSP* genes in cattle breeds from various regions around the world, research on native cattle breeds in Türkiye is limited (Öner et al., 2017; Atalay and Kök, 2023; Atalay et al., 2024). The objective of this study is to detect polymorphisms in the *HSP90AB1* (SNP g.4338T>C) gene in Turkish native cattle breeds namely Anatolian Black (AB), East Anatolian Red (EAR), and Turkish Grey Step (TGS), as well as commercial Holstein Friesian (HF) cattle. The results of this study are expected to serve as a foundation for conservation and breeding efforts for HF and certain local cattle breeds reared in Türkiye, particularly under environmental conditions that are likely to change in the near future due to the effects of global warming.

2. Material and Methods

2.1. Animal sampling and DNA extraction

A total of 122 blood samples were collected from indigenous (AB n=30, EAR n=29, TGS n=29) and cosmopolitan (HF n=34) cattle breeds, obtained via the jugular vein into vacutainer tubes containing anticoagulant. The samples were respectively obtained from village herds in Antalya, Erzurum, Balıkesir, and Erzurum provinces for AB, TGS, EAR, and HF breeds. Blood samples were collected from at least three different populations of unrelated animals for each breed. Total DNA was extracted from blood samples using the GeneJET Genomic DNA Purification Kit (Thermo K0721) following the manufacturer's recommendations.

2.2. AS-PCR amplification and genotyping

The polymorphism in the *HSP90AB1* gene (SNP g.4338T>C), associated with heat tolerance as reported by Sajjanar et al. (2015), was identified in this study using the Allele-Specific PCR method. For this purpose, the single nucleotide polymorphism (SNP-4338T>C) in the relevant gene region was identified through PCR using the primers provided in Table 1, which present the base sequences. Both mutant and normal alleles were simultaneously determined in the Allele-Specific PCR process by applying two different PCR procedures.

Table 1. Primers utilized in AS-PCR procedure for determination of *HSP90AB1* polymorphism

SNP	Primer code	Primer sequence (5' – 3')	Annealing Temperature (oC)	PCR Product Size
SNP4	HSP90 (Forward for C allele)	CTGGAGTCACACTGAGGAAC	62	560 bp
	HSP90 (Forward for T allele)	CTGGAGTCACACTGAGGAAT	62	
	HSP90 Common Reverse Primer	TGTTGGAGATCGTCACCTG	62	

PCR was performed in a 50 µL reaction volume, which included 50 ng of template DNA, 5 µL of 10X reaction buffer, 0.6 mM dNTPs, 2.5 mM MgCl2, 10 pM of each primer, 1 U of Taq DNA polymerase (GeNet Bio, Korea), and 31.25 µL nuclease-free water. PCR amplification began with an initial denaturation at 94 °C for 10 minutes, followed by 31 cycles of 40 seconds each at 94 °C, 62 °C, and 72 °C for denaturation, annealing, and extension, respectively. The final extension step was conducted at 72 °C for 10 minutes. All PCR products were separated on a 3.5% agarose gel to genotype individuals as CC, CT, and TT based on the presence or absence of PCR fragments.

2.3. Statistical analysis

GenAlEx software (Peakall and Smouse, 2012) was employed for calculating allele and genotype frequencies and for testing Hardy-Weinberg equilibrium (HWE) using the chi-square ($\chi 2$) approach. Calculation of allele and genotype frequencies involved directly counting the ratios of different alleles and genotypes, respectively, whereas the determination of the chi-square value was based on the utilization of the provided equation:

$$X^2 = \sum_{i=1}^{k} \frac{(O-E)^2}{E} \quad (1)$$

Where O and E represent the observed and expected number of animals of the i-th genotype.

3. Results and Discussion

Following AS-PCR analysis, it was observed that all examined breeds displayed polymorphism in the *HSP90AB1* gene region's SNP (SNP-4338T>C). The gene and genotype frequencies determined in the studied breeds are summarized in Table 2. The frequency of the C allele for the *HSP90AB1* (SNP-4338T>C) gene varied from 0.31 (TGS) to 0.62 (HF), whereas the frequency of the T allele ranged from 0.38 (HF) to 0.69 (TGS) across the investigated breeds. CC genotype frequency ranged from 0.00 (TGS) to 0.35 (HF), CT genotype frequency from 0.48 (EAR) to 0.62 (TGS), and TT genotype frequency from 0.12 (HF) to 0.38 (TGS) across all studied breeds (Figure 1). Notably, deviation from Hardy-Weinberg equilibrium was observed solely in the TGS population ($\chi 2 = 5.87$), while the other breeds remained consistent with Hardy-Weinberg equilibrium.

Table 2. *HSP90AB1* (SNP-4338T>C) gene, genotype frequencies, and chi-square values for animals

Breed	Sample Size	Alel frequencies		Genotype Frequencies			χ^2
		C	T	CC	CT	TT	
AB	30	0.37	0.63	0.10 (3)	0.53 (16)	0.37 (11)	0.660
EAR	29	0.55	0.45	0.31 (9)	0.48 (14)	0.21 (6)	0.017*
TGS	29	0.31	0.69	0.00 (0)	0.62 (18)	0.38 (11)	5.873*
HF	34	0.62	0.38	0.35 (12)	0.53 (18)	0.12 (4)	0.497

AB: Anatolian Black, EAR: East Anatolian Red, TGS: Turkish Grey Steppe, HF: Holstein Friesian.
χ^2 0.05;1: 3.84; *: Significant deviation from HWE.

Identifying candidate genes associated with heat tolerance is crucial to combat the potential negative impacts of climate change and ensure sustainability in farm animals. The use of these genes in MAS or Genomic Selection (GS) studies can lead to populations with high tolerance to heat stress. Heat Shock Protein (HSP) genes have emerged as key players in heat stress tolerance in farm animals (Demir et al., 2022).

Sajjanar et al. (2015) reported the CC, CT, and TT genotype frequencies for a polymorphic region (SNP g.4338T>C) on the *HSP90AB1* gene in 200 cattle (80 Sahiwal and 120 Frieswal) raised in India as 0.20, 0.70, and 0.10, respectively, for Frieswal cattle, and as 0.05, 0.78, and 0.17, respectively, for Sahiwal cattle. In addition, the authors highlighted that based on correlation analyses between these genotypes and certain thermo-physiological traits such as heat tolerance coefficient, average respiration rate, and average rectal temperature, the TT genotype is desirable for heat stress tolerance. Similar findings have been reported by Prastowo et al. (2021) in Holstein cattle raised in Indonesia. In the present study, the TT genotype frequency was determined as 0.37, 0.21, 0.38, and 0.12 in the AB, EAR, TGS, and HF breeds, respectively. The TT genotype frequency obtained in the HF breed is similar to the results reported for the Sahiwal and Frieswal breeds by Sajjanar et al. (2015). However, the frequency rates observed in Anatolian native cattle breeds (AB, EAR, TGS) in this study are higher than the genotype frequencies reported by Sajjanar et al. (2015) for the Sahiwal and Frieswal breeds. The underlying reason for this difference could be attributed to the genetic origins of these breeds. While the breeds utilized in this study (AB, EAR, TGS, and HF) have a genetic origin of B. taurus (Demir et al., 2023), Sahiwal and Frieswal breeds originate from B. indicus (Sajjanar et al., 2015).

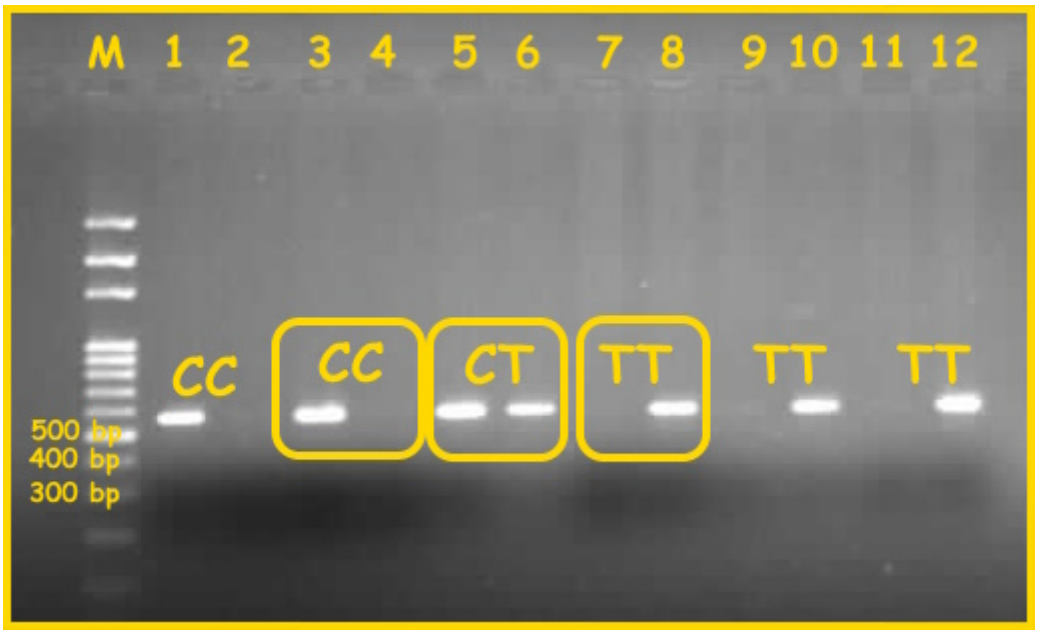

Figure 1. Agarose gel image of 560 bp length alleles amplified via AS-PCR M: Marker (Thermo 100 bp; Cat.No: SM0241). a) PCR prducts (1 % agarose gel) (560 bp).

In this study, it was observed that the frequency of the TT genotype within the EAR breed of native Turkish cattle breeds is lower compared to other local breeds. Considering the regions where these breeds are raised, these results are not surprising. The AB breed has the widest distribution among native Turkish cattle breeds. While it is raised in a large part of Anatolia, the samples of the AB breed used in this study were collected from the province of Antalya. Samples belonging to the TGS breed were collected from the province of Balıkesir. The average temperatures of the provinces where AB and TGS samples were collected are significantly higher than those of the province of Erzurum, where the EAR breed is raised. Therefore, it can be inferred that these two breeds have developed an adaptation to high temperatures over many years. The results obtained in the study conducted by Argun Karslı (2024) on the determination of polymorphism in the *HSP90AB1* (SNP g.4338T>C) region in Zavot, South Anatolian Yellow (SAY), and South Anatolian Red (SAR) cattle breeds support this view. Argun Karslı (2024) noted TT genotype frequencies of 0.47 and 0.35, respectively, in the SAY and SAR cattle breeds known for their adaptation to high temperatures, especially in the southern and southeastern Anatolia regions, which are recognized for Türkiye's high average temperatures.

Conclusion

The findings of this study highlight the importance of heat tolerance capacity in some Turkish local cattle breeds. These indigenous resources could be crucial for addressing the issues faced by climate change. The identification of polymorphisms associated with heat tolerance in cattle, such as *HSP90AB1*, could be a valuable tool in MAS studies. This may improve the adaptive capacity of Turkish local cattle and reduce the negative impacts of heat stress. The use of MAS techniques in Turkish native cattle shows promise for conserving their unique genetic characteristics and improving adaptation to future climate change scenarios. This could contribute to Türkiye's sustainable livestock and food security.

Ethical Statement

This research was approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (Protocol No: HAYDEK-931/2022).

Conflict of Interest

The authors declare that there are no conflicts of interest

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Author Contributions

S.Ö.U. was responsible for blood sample collection, laboratory analyses, and contributed to manuscript preparation and review. B.A.K. performed laboratory analyses and participated in drafting and revising the manuscript. Ü.B. contributed to data analyses, manuscript writing, and revisions. E.D. was involved in data analyses, manuscript drafting, and review. T.K. led the project, contributed to blood sample collection, data analyses, and was involved in manuscript drafting and critical review.

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