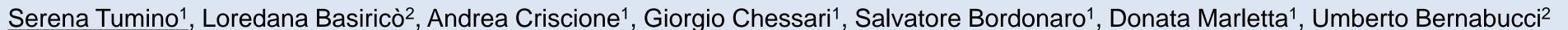
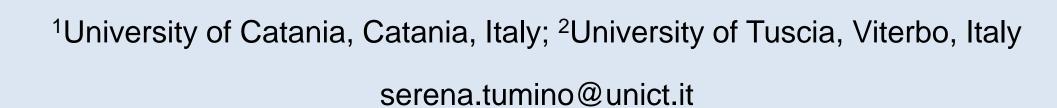
GENETIC VARIATION OF *HSP70.1* LOCUS IN CINISARA AND MODICANA CATTLE BREEDS







INTRODUCTION

Heat Shock Proteins (HSPs) are known to play crucial roles in environmental stress tolerance and thermal adaptation of animals. Among the HSP gene family, *HSP70.1* is believed to be temperature sensitive and induced by physiological, pathological, and environmental stressors. The present research aims to investigate the polymorphism of the *HSP70.1* gene (also known as *HSPA1A*) in two Sicilian native cattle breeds, Modicana and Cinisara, reared under hot climate conditions and, for comparison, in Italian Holstein.





MATERIAL AND METHODS 117 biological **Genotyping: DNA Data analysis** 4 SNPs extraction samples: 30 Modicana **30 Cinisara 57 Italian Holsten BTA 23** HSP70.1 gene 3'UTR 5'UTR **EXON** Promoter • g.895 C>-• g.2154 G>A • g.1128 G>T • g.64 G>T

RESULTS

Table 1. Genotype and allele frequencies at the 4 analyzed SNPs

SNP	Breed	Genotypes			All	Allele	
		(frequency %)			frequency (%)		
		CC	C-		С	-	
	Modicana	25 (83.34)	4 (13.33)	1 (3.33)	90	10	
5'UTR 895 C>-	Cinisara	19 (63,34)	10 (33.33)	1 (3.33)	80	20	
	Italian Holstein	47 (82.46)	9 (15.79)	1 (1.75)	90.35	9.65	
		TT	TG	GG	Т	G	
	Modicana	0	10 (33.33)	20 (66.67)	16.7	83.3	
5'UTR 1128 G>T	Cinisara	0	14 (46.67)	16 (53.33)	23.3	76.7	
	Italian Holstein	0	11 (19.30)	33 (57.89)	12.5	87.5	
		TT	TG	GG	Т	G	
	Modicana	0	3 (10)	27 (90)	5.08	94.92	
3'UTR.64 G>T	Cinisara	0	3 (10)	27 (90)	5.08	94.92	
	Italian Holstein 0	0	0	54 (100)	0	100	
		GG	GA	AA	G	A	
	Modicana	0	0	30 (100)	0	100	
3'UTR 2154 G>A	Cinisara	0	0	28 (100)	0	100	
	Italian Holstein	0	0	56 (100)	0	100	

Table 2. Frequencies of haplotypes at the HSP 70.1 locus in Sicilian cattle breeds

Modicana			Cinisara			
Haplotypes	Frequency	S.E	Haplotypes	Frequency	S.E	
GCG	0.760	0.013	GCG	0.709	0.016	
GCT	0.090	0.013	G-T	0.170	0.015	
G-T	0.076	0.013	GCT	0.051	0.004	
TCG	0.050	0.000	TCG	0.040	0.015	
G-G	0.024	0.013	G-G	0.018	0.004	

ACKNOWLEDGMENTS

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA-PNRR-MISSIONE 4 COMPONENTE 2. INVESTIMENTO 1.4 - D.D. 1032 17/06/2022. CN00000022).

All the SNP markers were found to be polymorphic in the Sicilian breeds, except for 3'UTR g.2154 G>A which was monomorphic for g.2154A as in Italian Holstein. Largely predominant alleles were observed at each locus in the whole sample, with frequencies of up to 95%, 80%, and 77% (at 3'UTR g.64G/T, 5'UTR g.895C/- and 5'UTR g.1128G/T, respectively) in the two Sicilian breeds. The 5'UTR g.895C/- locus was the most polymorphic, with three genotypes in all breeds. The deletion, associated with high expression of the HSP70 protein in response to heat stress, showed the highest frequency in Cinisara (Table 1). In both the Sicilian breeds, 5'UTR SNPs were found in *linkage* (r² 0.86), and the 5 inferred haplotypes showed a rather homogeneous distribution. In Table 2, all the haplotypes across the 3 polymorphic SNPs estimated using the fastPHASE software, which had a frequency greater than 1%, are presented, and distributed by population. Five haplotypes were observed for each Sicilian breed; the distribution appears fairly homogeneous, in terms of frequency ranking. The most frequent haplotype in the Sicilian breeds was GCG, with a frequency of 76% in Modicana and 71% in Cinisara, respectively. In terms of haplotype variability, the Cinisara breed exhibits a greater distribution of "minor" haplotypes, which together account for 28% compared to 24% in Modicana. The distribution of haplotypes with GCT and G-T also differs, as they switch positions between the two Sicilian breeds. The G-G haplotype is similarly underrepresented (2%) in both Sicilian breeds.

CONCLUSIONS

The observed genetic variation at the *HSP70.1* locus provides new insights into the genetic determinism of thermotolerance in these Sicilian native breeds.





