Bulgarian Journal of Agricultural Science, 29 (No 6) 2023, 1143–1148

Genetic diversity analysis of the Rhodope Shorthorn cattle breed based on SSR markers

Milena Bozhilova-Sakova[1*], Denis Viryanski[1], Atanaska Teneva[2], Tsvetoslav Koynarski[3], Elena Todorovska[4] and Ivona Dimitrova[2]

[1] *Agricultural Academy, Institute of Animal Science, 2232 Kostinbrod, Bulgaria*
[2] *University of Forestry, Faculty of Plant protection, 1797 Sofia, Bulgaria*
[3] *Trakia University, Faculty of Veterinary Medicine, 6000 Stara Zagora, Bulgaria*
[4] *Agrobio Institute, Department of Functional Genetics Cereals, 1164 Sofia, Bulgaria*
[*]*Corresponding author:* bojilova_milena@abv.bg

Abstract

Bozhilova-Sakova, M., Viryanski, D., Teneva, A., Koynarski, Ts., Todorovska, E. & Dimitrova, I. (2023). Genetic diversity analysis of the Rhodope Shorthorn cattle breed based on SSR markers, *Bulg. J. Agric. Sci., 29*(6), 1143–1148

The aim of the present experiment was to study the genetic diversity in 79 individuals from the Rhodope Shorthorn cattle breed, raised in the region of Smolyan, Bulgaria. For the purpose of the experiment was used a panel of 11 microsatellite markers. A total of 103 alleles were identified and 7 of them were population-specific. The allele number per locus varied from 6 (BM1824) to 14 (TGLA53), with a mean number of 9.36. The highest number of heterozygotes (72) was observed in locus TGLA227 (n = 79). The lowest number of heterozygotes (41) was detected in locus ETH10 (n = 79). Microsatellite markers used in the current experiment showed PIC from 0.52 (ETH10) to 0.88 (ETH3), with a mean value of 0.897. Observed heterozygosity (Ho) varied from 0.767 (locus ETH10) to 0.946 (INRA23). Expected heterozygosity (genetic diversity – He) varied from 0.205 in locus ETH10 to 0.819 in locus TGLA227. Heterozygosity for all investigated loci was H_o = 0.897 and H_e = 0.586. Effective allele number (N) was between 7 (ETH10, INRA 23) and 14 (TGLA 53). Estimated values (PIC, H_o, H_e, and MNA) showed that all studied markers were polymorphic. All tested loci were with high Polymorphic information content (>0.5) and H_o > 0.6.

Keywords: Rhodope Shorthorn cattle; Genetic diversity; Microsatellite markers; Polymorphism

Introduction

The loss of genetic diversity leads to a number of issues related to the conservation of the gene pool and difficulties of selection as well. In recent years, the efforts of scientists have focused on various methods for studying genetic resources in animal husbandry (Demir et al., 2019).

In livestock, molecular markers can be used to enhance the selection programs based on the productive traits of animals. The application of modern methods and models for genetic assessment of breeding values in cattle could significantly increase the breeding process. That is why the study of genetic resources is extremely important in the organization and management of selection programs (Khalil, 2020). Using SSR as direct markers could increase the accuracy of selection programs from 0.63 to 0.83 (Solberg et al., 2008).

The Rhodope Shorthorn cattle is one of the two indigenous cattle breeds found in Bulgaria today. The main area of distribution is the Rhodopes, mainly the regions of Krumovgrad, Momchilgrad, Zlatograd, Madzharovo, and Smoly-

an. Rhodope Shorthorn is the smallest cattle breed in Bulgaria. It is used for meat and dairy production. The information about the genetic structure of the breed could be very valuable.

In recent years, only a few similar studies have been performed in Bulgaria on other cattle breeds (Teneva et al., 2005; Teneva et al., 2007). The available information is insufficient. Therefore, the present study is necessary in order to maintain and enrich the genetic database.

The aim of the present work was to study the genetic diversity of the Rhodope Shorthorn cattle breed consisting of 79 individuals raised in the village area of Smolyan, Bulgaria, using a panel of 11 microsatellite markers.

Materials and Methods

Animals

For the purpose of the present study the research team studied a total of 79 animals from Rhodope shorthorn cattle breed, raised in Bulgaria. Blood samples were collected from each individual in vacuum tube containing EDTA. The samples were stored at -20°C until the next step of experimental work.

DNA extraction

Genomic DNA was extracted from whole blood using ExgeneTM Tissue SV (plus) (GeneAll) purification kit according to the manufacturer's instructions. The concentration and quality of DNA were identified by spectrophotometer and agarose gel electrophoresis.

PCR amplification

A panel of 11 microsatellite markers recommended for cattle paternity testing by ISAG (Hoffmann & Amos, 2004) was used (Table 1). Microsatellites were amplified using the "StockMarks for Cattle® Bovine Genotyping Kit" (Applied Biosystems Inc., Foster City, CA) in multiplex reactions according to the manufacturer's recommendations. PCR amplification was performed with thermal cycler EPPENDORF (PE, Applied Biosystems) under the following conditions initial denaturation 95°C/15 min, 31 cycles, denaturation 94°C/45 s, annealing 55–65°C/45 s, elongation 72°C/60 s and final elongation 72°C/10 min. The tested loci, primer sets, and allele range are presented in Table 1.

Fragment analysis

The fluorescent labeled PCR products were submitted to fragment analysis by capillary electrophoresis, with an automated sequencer ABI PRISM 310 (Applied Biosystems), using the GeneScan-350 ROX® Size Standard (Applied Biosystems), according to the manufacturer's specifications. The information about fragment sizes was automatically estimated by the GENESCAN ANALYSIS v.3.1. Software.

Table 1. Tested locus, chromosome localization, primers, allele length

№	Locus	Chromosome localization	Marker	Primer sequence (5' -> 3')	Length of alleles
1	ETH225 (D9S1)	9	M3	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	131-159
2	INRA023 (D3S10)	3	M9	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTC	195-225
3	ETH10(D5S3)	5	M10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	207-231
4	ETH3 (D19S2)	19	M14	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	103-133
5	BM2113(D2S26)	2	M15	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	122-156
6	BM1824(D1S34)	1	M16	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	176-197
7	TGLA227(D18S1)	18	M26	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	75-105
8	TGLA126(D20S1)	20	M27	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	115-131
9	TGLA122(D21S6)	21	M28	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	136-184
10	TGLA53 (D16S3)	16	M29	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	143-191
11	SPS115(D15)	15	M30	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCCTAGTTTGGCTGTG	234-258

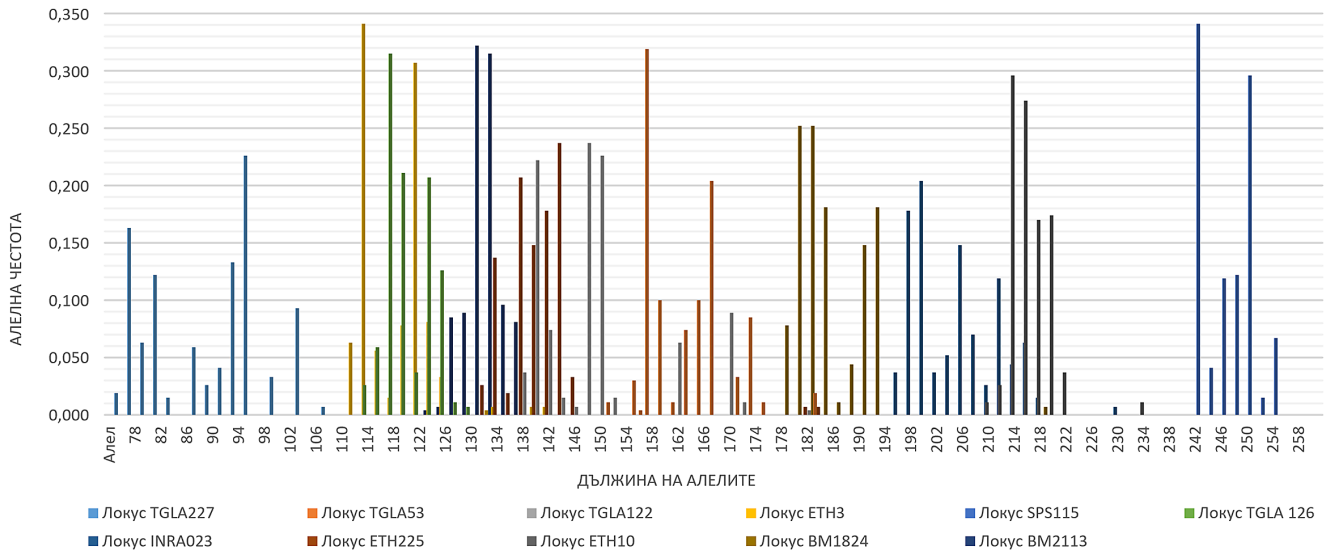

Fig. 1. Allelic frequency and length of alleles in Rhodope Shorthorn cattle estimated through 11 microsatellite loci

Statistical analysis

Genetic diversity of the tested animals was estimated based on allele frequencies, the mean number of alleles (MNA), observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC) by Powerstat v.1.2 Software (Figure 1).

Results and Discussion

In the present study in Rhodope Shorthorn cattle, a total of 103 alleles were identified in 11 microsatellite loci (Table 3). Seven of them were identified as population-specific, based on the relatively higher allele frequencies at some of the microsatellite loci: TGLA 53 – 1 allele, TGLA126 – 1 allele, ETH 10 – 2 alleles, BM1824 – 1 allele, BM2113 – 1 allele, SPS115 – 1 allele (Table 2). The allele number per locus varied from 6 (BM1824) to 14 (for TGLA 53) with a mean of 9.36. Allele frequency varied in different microsatellite loci. The loci with the highest observed allele frequency were SPS115 (0.646), ETH10 (0.525), and TGLA53 (0.335). The alleles with the lowest observed frequencies were found in loci TGLA122, TGLA53, ETH3, ETH225, BM1824, and BM2113 with values 0.006 (Table 2).

The highest number of heterozygotes (72) was observed in locus TGLA227 (n = 79), while the lowest number of heterozygotes (47) was established in locus SPS115 (n = 79). Microsatellite markers, used in the current experiment showed PIC from 0.52 (ETH10) to 0.88 (ETH3) with a mean of 0.75 (Table 3).

The observed heterozygosity (H_o) varied from 0.767 (locus ETH10) to 0.946 (INRA23). Expected heterozygosity (genetic diversity – H_e) varied from 0.205 (locus ETH10) to 0.819 (locus TGLA227) (Table 3). Mean heterozygosity was H_o = 0.897 and H_e = 0.586. Effective allele number (N) was between 7 (ETH10, INRA23) and 14 (TGLA53). Estimated values (PIC, H_o, H_e and Mean Number of Alleles) showed that all studied markers were polymorphic. All tested loci were with high PIC > 0.5 and H_o > 0.6 (Table 3). The total number of alleles, mean number of alleles, effective number of alleles, observed and expected heterozygosity are shown in Table 4.

In Bulgaria, similar studies were conducted by Teneva et al. (2005). The research team used the same 11 microsatellite markers to genotype 35 animals from Grey cattle from a herd reared in the region of Sredetz, Bulgaria. The authors reported a total of 83 alleles with an average number of 7.6 alleles per locus.

In a different study of 89 individuals from Rhodope and Grey cattle breed, Teneva et al. (2007) found 178 alleles in the same 11 loci – 118 alleles in Rhodope (R) and 60 in Grey (G) cattle with a mean of 16.2 alleles/locus. Their results showed that the Rhodope Shorthorn population had a greater mean number of alleles (9.0) than the Grey cattle (7.5), although this may have been due to the greater number of animals. The most polymorphic locus among the studied 11 microsatellite loci in both populations was TGLA 53, with each population having 13 alleles. In the present study, the same locus was identified with 14 alleles.

Table 2. Allele frequency, homo- and heterozygous variants of microsatellite loci in Rhodope Shorthorn cattle

TGLA 227		TGLA 53		TGLA 126		ETH3		SPS115		TGLA 122		INRA023		ETH 225		ETH10		BM1824		BM2113	
Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
81	0.095	155	0.184	117	0.057	115	0.07	240	0.013*	138	0.006*	175	0.025*	139	0.006*	213	0.019*	180	0.177	126	0.006*
83	0.038	161*	0.335*	119*	0.513*	117	0.089	248	0.646*	140	0.019*	178	0.013*	141	0.285	215	0.019*	182	0.247	128	0.146
85	0.152	163	0.025*	121	0.12	119	0.07	250	0.044	142	0.203	197	0.139	145	0.203	219	0.525*	184*	0.373*	130	0.013*
87	0.038	165	0.076	123	0.013*	125	0.108	252	0.076	144	0.297	199	0.063	147	0.032	221	0.361*	190	0.127	134	0.177
91	0.228	167	0.025*	125	0.038	127	0.025*	254	0.063	146	0.013*	205	0.152	149	0.222	223	0.051	191	0.006*	136	0.158
95	0.095	169	0.133	127	0.259	129	0.101	256	0.051	148	0.013*	207	0.108	151	0.057	225	0.013*	192	0.07	138	0.316
97	0.259	171	0.089			131	0.013*	258	0.025*	152	0.025*	209	0.127	157	0.006*	227	0.013*	Homozygotes – n = 12 / Heterozygotes – n = 67		140	0.133
101	0.07	173	0.013*			135	0.114	260	0.082	154	0.12	211	0.032	159	0.171	Homozygotes – n = 38 Heterozygotes – n = 41		Heterozygotes – n = 67		Homozygotes – n = 12	
103	0.025*	175	0.006*			137	0.108			156	0.032	213	0.222	161	0.019*						
		177	0.019*			139	0.177			164	0.051	215	0.089								
		179	0.013*			141	0.12			172	0.032	217	0.032								
		181	0.032			143	0.006*			176	0.114										
		183	0.038							178	0.076										
		185	0.013*					Homozygotes – n = 32 Heterozygotes – n = 47		Homozygotes – n = 12		Homozygotes – n = 10		Homozygotes – n = 9 Heterozygotes – n = 70							
									Heterozygotes – n = 67		Heterozygotes – n = 69										
Homozygotes – n = 7 Heterozygotes – n = 72		Homozygotes – n = 20 Heterozygotes– n = 59 Heterozygotes – n = 52		Homozygotes – n = 27		Homozygotes – n =10 Heterozygotes – n = 69															

Table 3. Polymorphic information content, heterozygosity and number of alleles in the Rhodope Shorthorn cattle

Locus	Length of alleles (bp)	PIC	Ho	He	N
TGLA 227	81–103	0.81	0.925	0.819	9
BM 2113	126–142	0.77	0.916	0.691	8
TGLA 53	155–185	0.80	0.933	0.504	14
ETH 10	213–227	0.52	0.767	0.205	7
SPS 115	240–260	0.54	0.787	0.285	8
TGLA 122	138–178	0.81	0.931	0.691	13
TGLA 126	142–174	0.81	0.938	0.527	10
INRA 23	175–219	0.85	0.946	0.742	7
ETH 3	115–143	0.88	0.939	0.742	12
ETH 225	139–161	0.76	0.906	0.767	9
BM 1824	180–192	0.71	0.884	0.483	6
Total					103
Mean		0.75	0.897	0.586	9.36

[1]PIC – Polymorphic information content; [2]Ho – observed heterozygosity; [3]He – expected heterozygosity; [4]N – number of alleles

Table 4. Estimated genetic diversity of studied animals from Rhodope Shorthorn cattle breed.

Breed	Number of alleles	Na	Ne	Ho	He	Fis	df	χ^2	P**
Rhodope Shorthorn	103	9.36	5.03	0.77	0.76	-0.01	42.63	92.43	0.086

[1]Na – Mean number of alleles; [2]Ne – number of effective alleles; [3]Ho – observed Heterozygosity; [4]He – expected heterozygosity; [5]Fis – inbreeding coefficient; [6] $\chi 2$ – Chi square test; [7]df – degree of freedom; [8] P- probability

The alleles with the highest expected heterozygosity (He) was in locus TGLA227 with a value of 0.898 (Teneva et al., 2007). The alleles with the lowest He were found in locus SPS115 – 0.542. In our study, the highest expected heterozygosity were obtained in locus TGLA227 with 0.819 which was the locus with the highest allele frequency observed in findings of Teneva et al. (2007).

Stevanovic et al. (2010), investigated the same group of SSR markers (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA023, ETH3, ETH225, BM1824) in the population of Yugoslav Pled cattle in Serbia. They tested a total of 40 individuals and determined 91 alleles. The mean allele number per locus was 8.273, which was close to our results in Rhodope shorthorn cattle – 9.36.

Shelyov et al. (2017), studied 45 animals from Ukrainian red-spotted and 43 animals from Ukrainian black-spotted (43 individuals), They have used 10 SSR markers (TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824, TGLA227, BM2113, ETH10, SPS115) and established the highest observed heterozygosity in locus BM2113 (0.866) and expected heterozygosity 0.862 of the Ukrainian red spotted. In Ukrainian black-spotted the highest Ho was observed in locus TGLA227 – 0.930 and He – 0.896. For comparison in the present study in the Rhodope shorthorn cattle for locus BM2113 Ho was 0.916 and He was 0.691 and for locus TGLA227 Ho was 0.925 and He – 0.819.

In a study of the genetic diversity of the Zimbabwean Sanga cattle, Gororo et al. (2018), use 16 microsatellite markers, 11 of which were the same used in the present experiment. The highest number of alleles was detected in locus TGLA122 – 11, while the same marker in the Rhodope Shorthorn breed was observed with 10 alleles.

In another study of 11 Pakistani native breeds (*Bos Indicus*), Hussain et al. (2016) used 21 microsatellite markers to establish their genetic diversity. They identified a total of 476 alleles. The largest number of alleles is observed in locus TGLA126 – 43 alleles, whose number is significantly higher than that found in our study of the Rhodope Shorthorn – 10 alleles.

Conclusion

The present study aimed to assess genetic diversity in Rhodope Shorthorn cattle. All 11 tested microsatellite markers were polymorphic according to the number of alleles and the Polymorphic information content (PIC). The largest number of alleles was established in locus TGLA53 – a total of 14 alleles. The mean number of alleles per locus was 9.36. Alleles with the highest frequency in the Rhodope Shorthorn were found in SPS115 (0.646), ETH10 (0.525) and TGLA53 (0.336). The microsatellite marker ETH3 – 0.88 had the highest PIC. Highest H_o – was observed in locus INRA23

– 0.946. Seven population-specific alleles were found: TGLA53 – 1 allele, TGLA126 – 1 allele, ETH 10 – 2 alleles, BM1824 – 1 allele, BM2113 – 1 allele, SPS115 – 1 allele.

Modern techniques for DNA analysis, such as microsatellite markers could be used to reduce the chance of inbreeding and to preserve the genetic diversity in populations. Therefore, we recommend further studies in this direction.

References

Agricultural Report (2020). Ministry of Agriculture, Food and Forestry https://www.mzh.government.bg/media/filer_public/2020/12/03/agd_2020_web.pdf.

Demir, E. & Balcioglu, E. (2019). Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers. *Czech Journal of Animal Science, 64*(10), 411–419.

Gororo, E., Makuza, M., Chidzwondo, F. & Sanyika, T. (2018.) Genetic diversity in Zimbabwean Sanga cattle breeds using microsatellite markers. *South African Journal of Animal Science, 48*(1), 128-141.

Hoffman, J. & Amos, W. (2004). Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol., 14*(2), 599-612.

Hussain, T., Babar, M., Peters, S., Wajid, A., Akhtar, A., Azam, A., Zahoor, A., Wasim, M., Ali, A., Kadir, K., De Donato, M. & Ikhide-Hussain, I. (2016). Microsatellite Markers Based Genetic Evaluation of Pakistani Cattle Breeds. *Pakistan Journal of Zoology, 48*(6), 1633-1641.

Khalil, M. (2020). Molecular applications of candidate genes in genetic improvement programs in livestock. *Egyptian Journal of Animal Production, 57*, 1-23.

Shelyov, A., Kopylov, K., Kramarenko, S. & Kramarenko, O. (2017) Analysis of population-genetic processes in different cattle breeds by microsatellite loci of DNA. *Agricultural Science and Practice,* 74-78.

Solberg, T., Sonesson, A., Woolliams, J. & Meuwissen, H. (2008). Genomic selection using different marker types and densities, *Journal of Animal Science, 86*(10), 2447–2454.

Stevanovic, J., Stanimirovic, Z., Dimitrijevic, V. & Maletic, M. (2010). Evaluation of 11 microsatellite loci for their use in paternity testing in Yugoslav Pied cattle (YU Simmental cattle). *Czech Journal of Animal Science, 55*(6), 221-226.

Teneva, A., Todorovska, E., Tyufekchiev, N., Kozelov, L., Atanassov, A., Foteva, S., Ralcheva, S. & Zlatarev, S. (2005). Molecular characterization of Bulgarian livestock genetic resources. I. Genetic diversity in Bulgarian grey cattle as revealed by microsatellite markers. *Biotechnology in Animal Husbandry, 21*(5-6), 35-41.

Teneva, A., Todorovska, E., Tyufekchiev, N., Stella, A., Boettcher, P. & Dimitrova I. (2007). Molecular characterization of Bulgarian livestock genetic resources. II. Microsatelite variation within and among Bulgarian cattle breeds. *Biotechnology in Animal Husbandry, 23*(5-6), 227-242.

Received: **November, 02, 2022**; *Approved*: **December, 06, 2022;** *Published:* **December, 2023**