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Genetic variability of 11 microsatellite markers of the Brown Cattle, reared in Bulgaria

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ABSTRACT

The genetic characteristics, based on microsatellites in Brown cattle breed reared in Bulgaria, are unknown. In the present study, 11 microsatellite (STR) markers, recommended by ISAG/FAO in cattle, were used for the first time to genotype and evaluate the genetic diversity in 52 animals from the Brown cattle population in Bulgaria. All of the studied loci were informative and polymorphic. In total, 77 alleles were detected with a mean number of 7.00 at 11 microsatellite loci, from which 27 (36%) were with a frequency lower than 5%. Allele frequencies ranged from 0.010 to 0.490. The highest number of alleles was found in TGLA227 and TGLA122 (11 alleles), while the lowest - in BM1824 and TGLA126 loci (4 alleles). Microsatellite markers used in this study showed PIC values from 0.58 (TGLA126) to 0.86 (TGLA227) with an average of 0.70.

The results from this study showed an appearance of homo- and heterozygous variants and their different distribution over the 11 microsatellite loci. The highest number of heterozygotes was observed in locus TGLA227 (n=52), in which almost all individuals (48) were heterozygous. The lowest number of heterozygous individuals (18) was found in locus BM2113 (n=52).

In our study, the observed heterozygosity (H_o) and expected heterozygosity (H_e) varied from 0.643 (locus TGLA 122) to 0.942 (TGLA 227), and from 0.078 (locus BM2113) to 0.880 (locus TGLA227), respectively. For all 11 loci, the average heterozygosity was H_o= 0,849 and He=0,449.

This study is basic information in undertaking a conservation program and/or for support in the elaboration of a genetic improvement program for the Bulgarian Brown cattle breed. This information is of importance for farmers to successfully design of breeding schemes to avoid inbreeding and to reduce the loss of some specific traits.

Key words: microsatellite markers, genetic diversity, Bulgarian Brown cattle, polymorphism

Introduction

During the recent two decades, molecular biology created valuable new means for studying cattle livestock genetics and breeding techniques the DNA established molecular markers that are based on the mutations of the nucleotide sequence within the individual's genome (Suh et al., 2014). The most widely used molecular tool for assessment of genetic diversity is microsatellite markers (SSRs) (Alvarez et al., 2004; Tapio et al., 2005; Ivankovic, 2005; Teneva et. al., 2005; Handley et al., 2007; Ligda et al., 2009; Bozzi et al., 2009; Hristova et al., 2017). Microsatellite markers are commonly used to evaluate genetic diversity within and among species and breeds because they are highly informative and conservative. Microsatellites have been effectively used to understand bovine domestication and migration pattern (Bradley et al., 1994; Loftus et al., 1994; Edwards et al., 2000) and to evaluate genetic diversity and relationships among cattle populations (MacHugh et al., 1997; Canon et al., 2001; Kim et al., 2002; Maudet et al., 2002; Dorji et al., 2003; Jordana et al., 2003; Metta et al., 2004; Mukesh et al., 2004: Pandey et al., 2004). In Bulgaria, microsatellites markers have been widely applied to study the genetic diversity in sheep (Hristova et. al., 2012; 2014; 2017) and cattle (Teneva et al., 2005; Teneva et al., 2007).

The use of local breeds is important for the achievement of local food security objectives. The necessity to extend, maintain, and conserve genetic diversity has been outlined (Teneva et al., 2007).

RFLP, STR, and SNP markers are bi-allelic markers, because of their maximal heterozygosity value of 0.5. Among

them, microsatellites are the most polymorphic and informative which makes them an "ideal" marker system for identification of individuals, especially in parentage analysis, diversity analysis, QTL mapping, etc. (Teneva et al., 2013).

Currently, polymorphisms in gene products such as enzymes, blood group systems, leukocyte antigens which have traditionally been used for population studies are being replaced by DNA markers (Teneva et al., 2005). The existence of the PCR method facilitates the amplification of certain DNA fragments of interest which could help in studying the population. Microsatellites are the most widely used molecular markers in pedigree control. The use of microsatellites with high polymorphism information content (PIC) would help to correctly identify individual cattle for better operation of cattle breeding programs (Cervini et al., 2006). Molecular markers are widespread across the genome and are effective in determining population substructures.

In recent years, significant changes in the population of the Brown Cattle breed were observed. They cover breed productivity, genetic diversity, and the changes of the breeding programs which affect the overall quality of the farmed animals. Brown cattle are the second most prevalent breed in Bulgaria. The Bulgarian brown cattle were created by reproductively crossing the local Gray Cattle with Brown Alpine and Brown American cattle. The breed was approved in 1981.

In 2018, the process of specialization of production in cattle breeding continues, and it is characterized by the transition from dairy to meat production. As of 01.11.2018, the total number of cattle in the country shrunk by 2.5% compared to a year earlier, which was 526 491 total animals. The number of dairy cows decreased by 7.1%, at the expense of the increasing number of meat-producing cows - 10.5%. Thus, the share of meat cows in the total number of cows reached 31.3% compared to 27.7% in the previous year (Bulgarian Agricultural Report, 2018).

Assessment of molecular genetic diversity is an important activity related to phenotypic characteristics and productive traits in livestock. In the present study, a set of 11 microsatellite markers was used to evaluate genetic diversity in the Brown cattle breed population. The SSR molecular characterization of the Brown cattle breed has been conducted for the first time in Bulgaria.

Materials and Methods

Breed description

The Brown cattle are most common in the mountainous regions of the Southwestern part (incl. Sofia region), the plains and mountainous areas of South and South-eastern Bulgaria, and the mountainous parts of Central North Bulgaria (Lovech and V. Tarnovo). Cattle of this breed have a dark brown to light brown colour coat, with lighter animals of more American Brown breed.

The brown breed in Bulgaria occupies about 12-13% of all cows, with a trend towards increased demand from farmers and an increase in its relative share. In recent years Bulgarian Brown Beef has been refined with American Brown Beef bulls.

Biological material

Blood samples were acquired from 52 randomly selected animals belonging to the Bulgarian Brown cattle breed from the farm in Vrana, Sofia region. **The cows are generations of artificial insemination, through donor semen, imported from selected bulls of the American Brown breed.**

DNA amplification and genotyping individuals

Blood was taken from vena jugulars in vacutainers containing EDTA as an anticoagulant.

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 checked with gel electrophoresis and spectrophotometer.

In this study, 11 microsatellite markers recommended by ISAG/FAO (Hoffmann et al., 2004) were applied (Table 1). The markers were amplified in multiplexes using Stocks marks Cattle Paternity PCR typing protocol (Perkin Elmer). PCR amplification was performed with thermocycler EPPENDORF (PE, Applied Biosystems) under the following conditions: initial denaturation 95^{o}C/15 min, 31 cycles, denaturation 94^{o}C/45 s, annealing 61^{o}C/45 s, elongation 72^{o}C/60 min and final elongation 25^{o}C/2h. After amplification, the PCR products were mixed with fluorescence dye FAM, JOE, or NED, according to their sizes. The amplified products were separated on 5 % long-range gel on ABI PRISM 3710 automated sequencer using a Gene Scan – 350 internal size standard labeled with a ROX dye. The information about fragment sizes was automatically estimated by the GENESCAN ANALYSIS v.3.1. Software.

The information concerning the locus name, localization, primer sequences, annealing temperature, and allele range of the investigated microsatellite markers is shown in Table 1.

Statistical methods

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

Results

The efficiency of amplification and genotype identification was very high. PCR products with the expected lengths were obtained in all 52 samples.

Table 2 presents the acquired results about allele frequency, homo- and heterozygote variants in the investigated loci.

In total, 77 alleles were detected in the 11 loci. The number of alleles per locus ranged from 4 (TGLA 126 and BM 1824) to 11 (TGLA 227 and TGLA 122) with a mean number 7.0.

The allele frequency varied in different microsatellite loci. The highest and the lowest established allele frequencies of the 11 microsatellite loci in this study are described in Table 2.

In locus TGLA227, the highest allele frequency was established for allele 93 bp (0.176), and the lowest for allele 89 bp (0.020).

In TGLA53, the highest allele frequency was observed for allele 159 bp (0.430), and the lowest one for allele 163bp and 175bp (0.020).

The locus TGLA 122 showed the highest frequency for allele 140 (0.363), and the lowest for allele 134, 152 and 182 (0.010).

The highest frequency was found for allele 113 (0.490), and the lowest for allele 125 (0.010) in the ETH3 locus.

Regarding locus BM2113, the highest allele frequency was established for allele 130 (0.304) and the lowest for allele 126 (0.020).

Concerning locus SPS115, the highest allele frequency was established for allele 245 (0.320) and the lowest for allele 247 (0.020).

For the locus TGLA 126, the highest allele frequency was found for allele116 (0.420) and the lowest for allele 124 (0.050).

In INRA23 locus, the highest allele frequency was established for allele 217 (0.392) and the lowest for allele 219 and 221 (0,010).

In the locus ETH225, the highest allele frequency was observed for allele (0.333), and the lowest for allele 152 (0.010).

The ETH10 showed the highest allele frequency for allele 215 (0.480), and the lowest for allele 213 bp (0.020).

In the locus BM1824, the highest allele frequency was established for allele 183 bp (0.350), and lowest for allele 185 bp (0.140).

Alleles with low (<0.05) frequency were observed in almost all studied loci. In total 27 alleles had frequency under 0.05 that means 36 % of alleles established for the population are rare. Among them, 8 alleles with very low frequency (0.010) were found and these were distributed in the following studied here microsatellite loci – TGLA122 (3 alleles), INRA023 (2 alleles), BM2113 (1 allele), ETH225 (1 allele) and ETH3 (1 allele).

The results from this study showed an appearance of homo- and heterozygous variants and their different distribution over the 11 microsatellites loci. The highest number of heterozygotes was observed in locus TGLA 227 (n=52) in which almost all individuals (48) were heterozygous. The lowest number of heterozygous individuals (18) was found in locus BM2113 (n=52) (Table 2).

Microsatellite markers used in this study showed PIC values from 0.58 (TGLA 126) to 0.86 (TGLA 227) with an average of 0.70. All PIC values were higher than 0.5 and they could be considered as informative in population-genetic analyses (Botstein et al., 1980).

Within population variation (diversity) was measured by estimation of the level of heterozygosity and the number of alleles from microsatellite data.

In our study, the observed heterozygosity (H_o) varied from 0.643 (locus TGLA 122) to 0.942 (TGLA 227). The expected heterozygosity (gene diversity - H_e) varied from 0.078 in locus BM 2113 to 0.880 in TGLA 227 locus. For all 11 loci, the average heterozygosity was respectively H_o= 0,849 and He=0,449.

The effective number of alleles (N) was between 4 (TGLA 126 and BM 1824) and 11 (TGLA 122 and TGLA 227).

The calculated values of genetic diversity parameters - PIC, H_o, H_e and MNA showed that all microsatellite markers were polymorphic (Table 3). All the examined loci were characterized by high (>0.5) polymorphic content (PIC) and observed heterozygosity (Ho>0.6).

Table 1. *Locus, localization, primer sets, annealing temperature and fragment sizes of investigated SSRs*

No	Locus name	Chromosome localization	Marker	Primer set	Annealing T C^0	Allele range
1.	ETH225 (D951)	9	M3	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	56-65	131-159
2.	INRA023 (3810)	3	M9	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTC	55	195-225
3.	ETH10	5	M10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	55-65	207-231
4.	ETH3	10	M14	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	55-65	103-133
5.	BM2113	2	M15	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	55-60	122-156

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6.	BM1824	1	M16	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	55-60	176-197
7.	TGLA227	18	M26	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	55-56	75-105
8.	TGLA126	20	M27	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	55-58	115-131
9.	TGLA122	21	M28	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	55-58	136-184
10.	TGLA23	16	M29	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	55	143-191
11.	SPS115	15	M30	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCCTAGTTTGGCTGTG	55-60	234-258

Table 2. *Allele frequency, homo- and heterozygote variants at TGLA 227, TGLA 53, TGLA122, ETH3, BM2113 SPS115, TGLA 126, INRA 23, ETH 225, ETH10, BM1824 microsatellite loci in Brown cattle.*

TGLA 227 n=52		**TGLA 53 n=52**		**TGLA 122 n=52**		**ETH3 n=52**		**BM2113 n=52**		**SPS115 n=52**		**TGLA 126 n=52**		**INRA23 n=52**		**ETH 225 n=52**		**ETH10 n=52**		**BM1824 n=52**	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
75	0.029	151	0.030	134	0.010	111	0.020	122	0.098	245	0.320	116	0.420	201	0.108	140	0.216	213	0.020	183	0.350
77	0.108	157	0.210	138	0.029	113	0.490	124	0.039	247	0.020	118	0.390	203	0.078	144	0.108	215	0.480	185	0.140
79	0.088	159	0.430	140	0.363	115	0.100	126	0.020	249	0.220	122	0.140	209	0.186	146	0.167	217	0.196	187	0.230
81	0.167	161	0.040	146	0.029	121	0.270	130	0.304	251	0.100	124	0.050	211	0.157	148	0.167	219	0.167	193	0.270
87	0.029	163	0.020	148	0.078	123	0.110	132	0.235	253	0.280			213	0.059	150	0.333	221	0.137		
89	0.020	165	0.110	150	0.294	125	0.010	134	0.137	257	0.060			217	0.392	152	0.010				
91	0.049	167	0.110	152	0.010			136	0.157					219	0.010						
93	0.176	173	0.030	154	0.059									221	0.010						
95	0.157	175	0.020	160	0.049																
99	0.029			170	0.069																
103	0.147			182	0.010																
Homozygotes n=4 Heterozygotes n=48		Homozygotes n=27 Heterozygotes n=25		Homozygotes n=9 Heterozygotes n=43		Homozygotes n=22 Heterozygotes n=30		Homozygotes n=34 Heterozygotes n=18		Homozvgotes n=14 Heterozygotes n=38		Homozygotes n=23 Heterozygotes n=29		Homozygotes n=17 Heterozygotes n=35		Homozygotes n=9 Heterozygotes n=43		Homozygotes n=16 Heterozygotes n=36		Homozygotes n=13 Heterozvgotes n=39	

Table 3. *Polymorphic information content, heterozygosity and number of alleles in Brown cattle*.

Locus	Observed allele size range	PIC	Ho	He	N
TGLA 227	75-103	0.86	0.942	0.880	11
BM 2113	122-136	0.77	0.878	0.078	7
TGLA 53	151-175	0.71	0.868	0.171	9
ETH 10	213-221	0.64	0.856	0.407	5
SPS 115	245-257	0.72	0.896	0.460	6
TGLA 126	116-124	0.58	0.786	0.268	4
TGLA 122	134-182	0.73	0.643	0.818	11

INRA 23	201-221	0.74	0.900	0.407	8
ETH 3	111-125	0.61	0.828	0.246	6
ETH 225	140-152	0.74	0.884	0.681	6
BM 1824	183-193	0.68	0.862	0.527	4
Total		7.78			77
Mean		**0.70**	**0.849**	**0.449**	**7.00**

Conclusion

The present study is the first report on the genetic diversity assessment of Bulgarian Brown cattle breed using STR markers. The investigation confirmed the high polymorphism of the selected microsatellite markers and their application in the determination of the genetic structure of cattle populations. This study gives basic information in undertaking a conservation program and/or for support in the elaboration of a genetic improvement program for the Bulgarian Brown cattle breed.

The information can be used by farmers in different breeding programs to avoid inbreeding which can result in the loss of some specific traits and effective management of farm animal genetic resources (FAnGR) that requires comprehensive knowledge of the breed characteristics, including data on population size and structure, and within and between breed genetic diversity. The integration of these different types of data will result in the most complete representation possible of biological diversity within and among breeds, thus it will facilitate effective management of FAnGR.

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