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The polymorphism of *Rse*I type at *STAT 5A* locus in cattle

Teodora C. Carşai, Augustin Vlaic

Department of Animal Genetics, Faculty of Animal Husbandry and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
Corresponding author: A. Vlaic, vlaic.augustin@usamvluj.ro

Abstract. The association between the polymorphisms at different gene loci and milk traits is studied in many cattle breeds. Our study aimed to perform genotypization at *STAT 5A* locus in Romanian Black Spotted cattle (BNR = Bălțată cu Negru Românească), individuals with big milk production (BNR-M) and small milk production (BNR-S), in order to identify the gene and allele frequency. In order to perform the genotypization at *STAT 5A* locus in cattle, specific primer sets were used (some described by literature, other new synthesized). They allowed the amplification of exons from genes where mutations were identified. The genetic analysis was performed using PCR-RFLP. For each amplification products possible to be obtained from polymorphic regions, based on mutations, there were performed theoretic restriction maps with NEBcutter V2.0. The results of our study show that CT genotype and allele C were identified only in BNR-M genotype.
Key Words: Polymorphism, *STAT 5*, cattle.

Résumé. L'association entre le polymorphisme au locus des différents gènes et lait traits est étudiée dans nombreuses races de bovins. Notre étude visait à effectuer genotipization au locus *STAT 5A* en Bétail Roumain Tacheté de Noir (BNR = Bălțată cu Negru Românească), individues avec grande (BNR-M) et petite production de lait (BNR-S), afin d'identifier la fréquence des gènes et des allèles. Afin d'effectuer la genotipization à *5 a STAT* locus chez les bovins, les amorces spécifiques étaient utilisés (certains cité par littérature, autre nouvelle synthétisée). Elles ont permis l'amplification de l'exons de gènes où les mutations ont été identifiées. L'analyse génétique a été réalisée à l'aide de la PCR-RFLP. Pour l'amplification de chaque produits possibles à partir des régions polymorphes, en fonction des mutations étaient des cartes, on a effectué de mapes de restriction théorique avec NEBcutter V2.0. Les résultats de notre étude montre que le génotype CT et l'allèle C ont été identifiés seulement dans le génotype de la BNR-M.
Key Words: Polymorphismes, *STAT 5*, bétail.

Introduction. Lot of literature mentions the association between the polymorphisms at different gene loci and milk traits in cattle (Arranz et al 1998; Blott et al 2003; Carşai & Bâlteanu 2010ab; Carşai et al 2011ab; Dybus 2002; Herrington & Carter-Su 2001; Huang et al 2008; Siadkowska et al 2006; Sun at al 2009; Viitala et al 2006). At *STAT 5* gene locus, Brym et al (2004) studied a G9501A type polymorphism from the 9 intron in Jersey. They found the association between the GG and a bigger milk quantity and a bigger fat percent, while the AA and AG were associated with bigger protein content. Flisikowsky et al (2004) studied a T12743C type polymorphism from the 16 exon in Polish Friesian. The TC genotype was associated with a bigger milk quantity and a bigger percent of dry matter, protein and lactose, compared to TT genotype. Sadeghi et al (2008) studied the same genotype Italian Holstein, and found that CT genotype is associated with a bigger protein quantity in milk. Selvaggi et al (2009) studied a C6853T type polymorphism from exon 7, and found that CC genotypes were associated with a bigger milk quantity and a bigger protein percent compared to CT and TT.

Material and Method

Sampling. The precise identification of the genetic structure at studied locus in cattle needed harvesting of big number of samples. As much as 120 blood samples were harvested from cattle with big (BNR-M) and small milk production (BNR-S). The blood

samples (2 mL/individual) for DNA extraction were harvested from a number of 24 Romanian Black Spotted cattle. The sterile sampling was performed from jugular vein in vacutainer tubes with K3-EDTA anticoagulant, and then stored at 4° C.

Aa much as 100 µL of each blood sample were transferred in a sterile 1.5 mL Eppendorf. A quantity of 600 µL PBS sterile solution was added to each sample, they were shortly vortexed, then centrifuged at 14,000 g for 20 seconds. The supernatant was removed each time, and washing performed two times. After final washing, and supernatant removal, the pellet containing white figurate elements was submitted to alkaline lysis with A solution (200 mM NaOH). After short centrifugation the tubes were incubated 15 minutes at 95^0C on termomixer.

The sample neutralizing was performed by adding an equal share of B solution (200 mM HCl + 100 mM Tris HCl- pH = 8.5). After short centrifugation and vortexing, the samples DNA quantity, purity, and concentration was quantified with Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) and by migration in agarose gel. The samples were diluted to a final concentration of 150 ng/µL, then stored at 4^0C in order to perform the genetic analysis at studied loci.

The design of the pairs of primers needed for amplification and sequencing of the regions codifying in GenBank (http://www.ncbi.nlm.nih.gov/). The primers were designed from the intron regions flanking the exons, using Primer 3 design programme (http://frodo.wi.mit.erifugat/primer3/). In primer pairs design and selection, the following aspects were taken into account: to flank the exonic regions of interest in a manner which allows the possibility to analyze the entire codifying region of the studied genes, and as consequence to find the possibility of identifying all possible polymorphisms; the alignment temperature to be as close as possible to 60^0C; the guanine and cytosine content to be minimum 40%; avoiding, as much is possible, to obtain unspecific products, or dimmers. The primer sequences are: P1-agccctacagctccaatcct -3', P2 - gggtgtacccgctgcttag -3'.

The amplification was performed with 2X Tissue Green Master kit (Fermentas, Vilnius, Lituania), 100 ng genomic DNA, extracted from studied cattle, 10 picomols of each forward, primer and reverse primer, respectively, using the following conditions of amplification: one cycle at 94°C/3 minutes, followed by 35 cycles at 94°C/30 seconds, 58°C/30 seconds, 72°C/60 seconds and a cycle of final extension 72°C/3 min.

The amplification products were analyzed by migration in 2% agarose gel, containing 1X SybrSafe (Invitrogen, Eugene, OR, USA). The electrophoresis was performed in TBE 1X (pH= 8.5), at constant voltage 65 V for 2 hours. The gel image was taken and analyzed with image acquisition system Molecular Imager Gel Doc XR System (BioRad Laboratories, Hercules, CA, USA).

In order to perform the genotypization at *STAT 5A* locus in cattle, specific primer sets were used (some described by literature, other new synthesized). They allowed the amplification of exons from genes where mutations were identified. The genetic analysis was performed using PCR-RFLP. For each amplification product (possible to be obtained from polymorphic regions based on mutations) a theoretic restriction map was performed with NEBcutter V2.0 (http://tools.neb.com/NEBcutter2/). The analysis of the theoretical restriction profiles allowed us to establish the aimed restriction enzymes and digestion profiles, useful for identification and correction of genotypes. This allowed the genotypization of all studied individuals.

Genotypization. For genotypization, all reactions of amplification of the target regions from the genes of interest were performed in the same conditions; using the MyTaq Red Mix, 2x kit (Bioline Reagents Ltd., London, UK), 100ng de genomic DNA, 10 picomols of each specified primer of the target region (forward, and reverse, respectively), in the following conditions of amplification: 1 cycle at 94°C/3 minutes, followed by 35 cycle at 94°C/45 de seconds, 58°C/45 seconds, 72°C/45 seconds and a cycle of final extension at 72°C/3 minutes.

For genotipization at *STAT 5A* locus a 281 bp product containing part of the intron 15, entire exon 16 and part of the intron 16 was amplified with specific primers. The restriction of the 281 bp amplification products was performed with: 12 µL PCR product, 20µL digestion mixture/sample containing 1 µL *RseI* fast digest enzyme, 2 µL fast digest

reaction buffer and 17 μL miliQ sterile whours, then analyzed in 3% agarose gel containing 1X Sybr Safe (Invitrogen, Eugene, OR, USA), in migration conditions described for cDNA.

Results and Discussion. In the STAT 5A gene locus, the presence of a timine/cytosine (T/C) type substitution is located on the restriction site of the enzyme *Rse*I from the exon 16. The digestion of the 281 bp amplified fragment (Figure 5A) two, of three possible, restriction profiles were emphasized (Figure 5B).

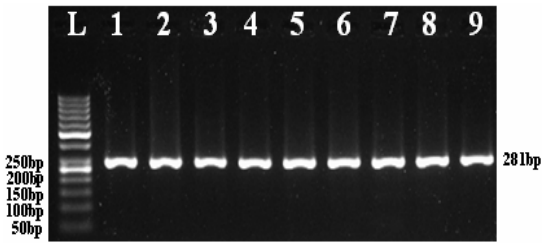

Figure 5A. Electrophoretic profile emphasizing the 281 bp products obtained from amplification of a region from the exon 16 of the *STAT 5A* gene, where is located the T/C type polymorphism; L: 50 bp DNA ladder (Fermentas, Vilnius, Lithuania).

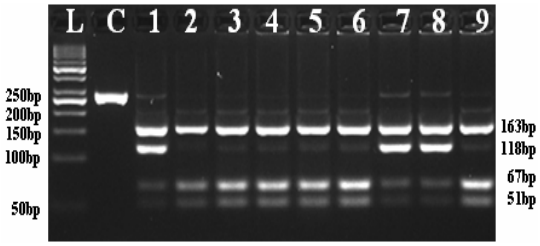

Figure 5B. Electrophoretic profile emphasizing the *RseI* type polymorphism, located on 16 exon of the *STAT5A* gene; L: 50 bp DNA ladder (Fermentas, Vilnius, Lithuania); C - PCR product not digested 281 bp control; fields: 1, 7 and 8 - TT genotypes; fields 2, 3, 4, 5, 6 and 9 - CT genotypes.

The 281 bp fragment amplified from the allele T contains two restriction sites for the *RseI* enzyme. After restriction, resulted three fragments of 163 bp, 67 bp, and 51 bp respectively, characteristic for the allele T. The T/C type restriction cancels one of the restriction sites for this enzyme from the amplified fragment. After restriction resulted only two fragments of 163 bp, and 118 bp respectively, characteristic for the allele C.

Analysis of the electrophoretic profiles emphasized two genotypes in individuals from the BNR-M group: homozygous genotypes of TT type, corresponding to the digested fragments of 163 bp, 67 bp, 51 bp and respectively, and heterozygous CT of type, which besides the tree emphasized fragments have a supplementary one of 118 bp. In BNR-S only TT genotype was emphasized. The homozygous genotype of CC type, characterized by the presence of two fragments of 163bp and 118bp, was not identified in studied breeds.

The individuals from the BNR-M group have almost double frequency of the TT type compared to CT genotype, while the frequency of the genotype CC was 0. The biggest frequency was recorded for the TT genotype in BNR-S and control individuals (M) with small milk production (Table 1).

Table 1

The genetic structure at *STAT 5A* locus in Romanian Black Spotted Cattle (BNR-M and BNR-S)

Breed (group)	Number of individuals genotyped using PCR-RFLP	Genotype frequency			Allele frequency	
		TT	CT	CC	T	C
BNR-individuals with big milk production (BNR-M)	60	0.636	0.364	0	0.818	0.182
BNR- individuals with small milk production (BNR-S)	60	1	0	0	1	0
M-control with small milk production	60	1	0	0	1	0

In BNR-M individuals, the observed frequency of the T allele was bigger compared to the frequency of allele C (which also has big frequency). In BNR-S individuals the observed frequency of allele T was 1, allele C being not identified.

Conclusions. In our study, the CT genotype and allele C were identified only in BNR-M genotype. The presence of a favorable allele in a certain locus does not guarantee a better quantitative and qualitative milk production, because of the presence of other alleles, with antagonist effects, in other loci.

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Authors:
Teodora Crina Carşai, Department of Animal Genetics, Faculty of Animal Husbandry and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur, Cluj-Napoca 400372, Romania; e-mail: teodora_carsai@yahoo.com
Augustin Vlaic, Department of Animal Genetics, Faculty of Animal Husbandry and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur, Cluj-Napoca 400372, Romania; e-mail: vlaic.augustin@usamvluj.ro

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