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# GENETIC VARIATION IN TWO BULGARIAN DOMESTIC GOAT BREEDS AS DETECTED WITH ISSR MAKERS

*Atanas Vuchkov<sup>1</sup>, Milena Kostova<sup>2</sup>, Bojin Bojinov<sup>2</sup>*

**Abstract:** The issue of conservation of genetic resources in livestock is of growing interest in recent decades. Local breeds of farm animals are usually with relatively lower productivity but well adapted to the specific conditions of the environment.

Due to the advent of a number of highly intensive selected breeds the existence of local genetic resources is threatened in our country. The risk comes from reducing the size of local populations of purebred animals, leading to narrowing of genetic diversity. Two local goat breeds with distinctive exterior, stably transmitted in the progeny were selected for the present study and their within-breed genetic variation assessed with ISSR markers.

**Key words:** ISSR markers, genetic diversity, local breeds, goats

## Introduction

The issue of conservation of genetic resources in livestock is of growing interest in recent decades. Local breeds of farm animals are usually with relatively lower productivity but well adapted to the specific conditions of the environment, and have increased resistance to multiple diseases specific to the region.

Due to the advent of a number of highly intensive selected breeds the existence of local genetic resources is threatened in our country. The risk comes from reducing the size of local populations of purebred animals, leading to narrowing of genetic diversity. For example, a number of local breeds of sheep – Rilomanastirska, Svishtovska and Panagyurska are already considered extinct.

Currently, Bulgaria has a relatively large gene pool with respect to local genetic resources in livestock - 17 local breeds of sheep, 2 local goat breeds, two local breeds of cattle, and 1 native horse breed. For some of these breeds the extinction risk is still real.

Local goats in Bulgaria have rarely been the subject of extensive studies. Despite scarce research in the past the zootechnical community in the country have been of the opinion that local goats are less productive, which was highlighted as a motive for crossing them with Saanska breed, the Czech white and the German white noble (Kadijski, 1958; Balevska and Tiankov 1971; Solomon et al., 1984).

This led to decreasing interest in local goat breeds. As a consequence of this lower interest the local goat population in Bulgaria gradually decreased. Only in the last 15-20 years there has been some reoccurring interest in the preservation of local goat breeds, both by farmers and by researchers.

Two local goat breeds with distinctive exterior, stably transmitted in the progeny – Kaloferska long-haired goat and Bulgarian Vitoroga long-haired goat (Vuchkov and

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Dimov, 2011; Vuchkov et al., 2011; Sedefchev et al., 2011) were selected for the present study.

In the past, both local breeds are clearly defined by their area of distribution - Kaloferska long-haired goat is bred mainly in villages in the foothills of the Central Stara Planina and Bulgarian Vitoroga long-haired goat – mainly in the mountains of southwestern Bulgaria. Today we are witnessing a process of "blurring" of the boundaries of the original (natural) areas of distribution of these two breeds. There is an increased interest from farmers from Blagoevgrad region in Kaloferska long-haired goat.

As a result of selective breeding with males and females from Kalofer region many purebred herds of this local breed were established in the Blagoevgrad region. Furthermore, at present the majority of the existing population of Kaloferska long-haired goat is located in southwestern Bulgaria.

In the 1960s team of Prof. D. Altman gathers a group of local long-haired goat from Southwest Bulgaria in order to preserve them in Germany as the original genetic material (Schuman, 2001). Thus, Germany formed a purebred population of Bulgarian vitoroga long-haired goats which are kept for 40 years in isolation as a pure form. In the course of many years of controlled breeding of this Bulgarian indigenous goat by German breeders it proved as stably transmitting its genotype in many generations. This proves that it is an indigenous breed with established genotype. Interestingly, no signs of inbred depression in this limited and isolated for long periods population is identified (Schuman, 2001).

The region of Southwest Bulgaria is the natural area of the other local breed – Bulgarian Vitoroga long-haired goat. This indigenous breed is preserved in its natural habitat today. Phenotypically and behaviorally it is quite different from Kaloferska long-haired goat (Vuchkov et al., 2011).

Kaloferska long-haired goat and Bulgarian vitoroga long-haired goat are available as relatively small populations. Coexistence and breeding of both goat populations as purebred lines, requires them to be clearly differentiated phenotypically and genotypically. This means determining the genetic distance between them and the genetic diversity within the two populations is essential.

Establishing of genetic diversity in the population is an essential first step for sustainable conservation of valuable genetic resources under threat of extinction. At present, genetic research on local goat populations has not been done and therefore valuable information is missing.

It is for the above reasons that we selected these two local goat breeds for studying their within-breed genetic variation with ISSR markers.

## **Material and methods**

### *Extraction of genomic DNA*

Experiments were carried at the laboratory of Molecular biology of the Department of Genetics and Plant Breeding, Faculty of Agronomy, Agricultural University of Plovdiv. The choice of source material for extraction of genomic DNA was made so as to apply non-invasive sample collection and to avoid any stress to the animals.

Isolation of genomic DNA from hair follicles of the Kaloferska long-haired breed and Bulgarian vitoroga long-haired breed was done with innuPREP DNA Kit (AnalyticaJena). Manufacturer protocol was followed and the steps were optimized for the conditions of our lab. Visualisation of the isolated genomic DNA was done after electrophoretic separation of the products in 1% agarose gel. Five  $\mu\text{l}$  of the final solution were applied to every slot from the extracted DNA and the staining was performed with ethidium bromide.

#### *ISSR (Inter-Simple Sequence Repeats) analysis*

ISSR analyses were performed by PCR reactions in QB-96 Thermal Cycler (Quanta Biotech, London, UK). Total levels of polymorphism were evaluated from produced multilocus anonymous dominant markers. Sequences of ISSR primers used to perform PCR reactions are listed in Table 1.

Table 1. Sequences of ISSR primers used to perform PCR reactions.

Primer name	DNA sequence	Length (bp)	Calculated melting temperature ( $^{\circ}\text{C}$ )
E7	AG(8)CTG	19	48.2
Tom14	AG(8)YT	18	51.4
Tom 11	GA(8)YC	18	53.9
Tom 5	CT(8)RG	18	53.9

PCR reactions were performed in 25  $\mu\text{l}$  reaction volumes, where for each reaction PCR buffer – 2.5  $\mu\text{l}$ ; dNTPs – 1.5  $\mu\text{l}$ ; ISSR primer – 1.5  $\mu\text{l}$ ; Taq – 0.12  $\mu\text{l}$ ;  $\text{H}_2\text{O}$  – 18.38  $\mu\text{l}$ ; 1  $\mu\text{l}$  genomic DNA were used.

ISSR PCR reactions were performed under the following regime: denaturing –  $94^{\circ}\text{C}$  for 3 min; 40 cycles of:  $94^{\circ}\text{C}$  – 1 min, primer melting temperature – 30 sec, elongation at  $72^{\circ}\text{C}$  – 45 sec, followed by a final extension at  $72^{\circ}\text{C}$  – 4 min, where primer melting temperature for each primer is calculated according to Kochieva et al. (2002).

#### *Statistical analysis*

Molecular data gathered throughout the current study was used for calculating relative genetic distances and producing hierarchical clusters with the “SPSS for Windows” statistical package.

## **Results and discussion**

#### *Extraction of genomic DNA*

For the purposes of ISSR analysis genomic DNA from the two goat genotypes was isolated with the standard innuPREP DNA Kit (AnalyticaJena), according to the supplier recommendations. Isolating sufficient quantity with a good quality genomic DNA was achieved when 15 hair follicles were used (Figure 1), which allowed for successful ISSR analyses later on. In general 300 to 500  $\mu\text{g}$  of genomic DNA were obtained as a

result. When the quantity or the quality of obtained DNA was considered insufficient DNA extraction procedure was repeated till adequate results were obtained.

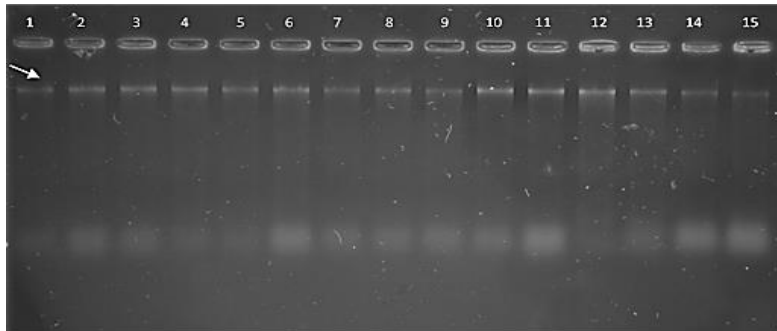


Figure 1. Results from the extraction of genomic DNA from hair follicles with innuPREP DNA Kit (arrow points to the position of genomic DNA fragments)

#### *ISSR analysis*

Initial screening with pre-selected ISSR markers (Fig. 2) was aimed at verifying the capacity of the selected marker system to reveal sufficient polymorphism within the two breeds. As demonstrated on Fig. 2 using the selected ISSR primers produced a number of polymorphic bands between individual animals. Therefore they were used to screen the two populations with the aim of revealing the genetic diversity both within and between the two local goat breeds.

As expected the use of different ISSR primers led to revealing different number of polymorphic bands in the two breeds. As a result of screening several primers and optimizing the PCR conditions 2 ISSR primers were identified that produce informative polymorphisms in these local breeds. Furthermore the polymorphisms revealed in each breed were sufficient to produce preliminary grouping of the animals within the two breeds (Fig. 3 and Fig. 4). The two dendrograms demonstrate both the capacity of the

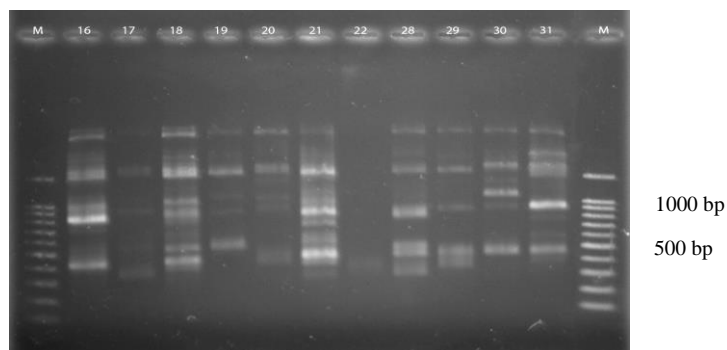


Figure 2. Polymorphisms within Kaloferska long-haired breed revealed with primer E7. Line M – 1kb DNA ladder. Numbered lanes – PCR products from different animals.

selected system to identify informative polymorphisms in long-haired goats and the need to further produce more polymorphic products so that adequate characterization of the individual animals can be obtained. The use of the 2 ISSR primers resulted in producing 12 polymorphic bands that were sufficient to reveal a group of 14-15 individuals with high level of genetic similarity in both breeds. These groups are clearly seen as uniform groups at the lower end of both Fig. 3 and Fig. 4. This apparent homogeneity however is due to the relatively low numbers of polymorphic bands produced so far. As a result these genotypes so far can not be distinguished and therefore individual animals selected for the breeding purposes. The application of the ISSR marker system however reveals its potential for revealing informative polymorphisms in local long-haired breeds. We aim to continue screening more primers so that sufficient polymorphisms can be obtained in the least number of PCR reactions.

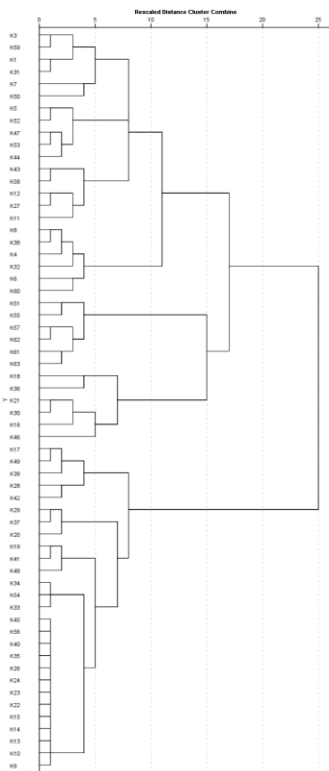


Figure 3. Grouping of animals from Kaloferska long-haired breed based on polymorphisms revealed with E7 and Tom 14 ISSR primers.

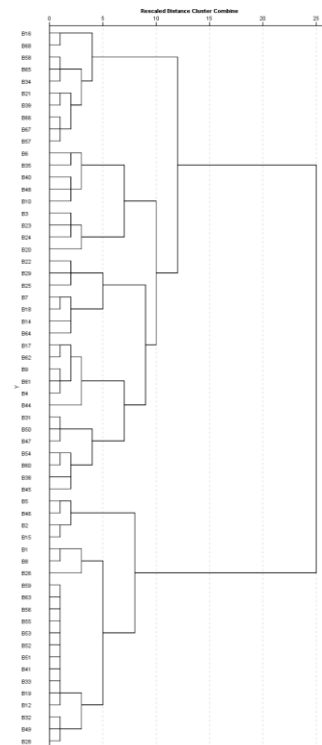


Figure 4. Grouping of animals from Bulgarian vitoroga long-haired breed based on polymorphisms revealed with E7 and Tom 14 ISSR primers.

## Conclusion

Despite the relatively small size and long inbreeding history in both local breeds significant diversity as established by molecular markers was revealed.

Conducted molecular analyzes revealed that the ISSR marker system is able to identify significant genetic diversity among the breeds.

Data accumulated so far is insufficient to adequately characterize each individual animal which makes continuation of the present work mandatory.

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