Bulgarian Journal of Agricultural Science, 24 (No 6) 2018, 1109–1113

Application of ISSR markers for detection of genetic variation in two Bulgarian autochthonous goat breeds

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Abstract

Kostova, M., & Bojinov, B. (2018). Application of ISSR markers for detection of genetic variation in two Bulgarian autochthonous goat breeds. *Bulgarian Journal of Agricultural Science*, *24*(6), 1109–1113

The conservation of genetic resources in livestock is of rising interest in recent years. The introduction of highly intensive selected breeds results in reducing the size of local ones, leading to narrowing of their genetic diversity. Therefore, estimating the remaining genetic variation is becoming of key importance for prioritizing conservation efforts and developing future breeding programs. In this respect, two local goat breeds with a distinctive exterior, stably transmitted in the progeny, were selected for the present study and their within-breed genetic variation assessed with ISSR markers. Our results demonstrate that ISSR markers are capable of revealing substantial genetic diversity in the autochthonous Bulgarian goat breeds. Furthermore, unique genetic profiles were obtained for each of the studied animals, thus providing usable tool for paternity testing in future breeding programs.

Keywords: ISSR markers; genetic diversity; local breeds; goats

Introduction

The conservation of genetic resources in livestock is of rising interest in recent years. While local breeds of farm animals are usually with relatively lower productivity they are well adapted to the specific conditions of the environment and have increased resistance to multiple diseases specific to the region.

The introduction of highly intensive selected breeds in modern animal husbandry threatens the existence of local genetic resources in Bulgaria. The threat arises from reducing the size of local populations, therefore narrowing the available genetic diversity. For example, a number of local breeds of sheep (i.e. Rilomanastirska, Svishtovska and Panagyurska) are already considered extinct. As a consequence, estimating of remaining genetic variation is becoming of key importance for prioritizing conservation efforts.

Currently, Bulgaria has a relatively large gene pool with respect to local genetic resources in livestock. These include 17 local breeds of sheep, 2 local goat breeds, two local breeds of cattle, and 1 native horse breed. However, for some of these breeds, the extinction risk is either remaining or even increasing.

Extensive studies of local goats in Bulgaria have rarely been performed. Despite scarce research general perception among breeders in our country is that local goats are less productive. In the past, this was used as a motive for crossing them with several more intensive breeds like Saanen, the Czech white and the German white noble (Kadijski, 1958; Balevska and Tiankov, 1971; Solomonov et al., 1984). The development of more productive breeds has led to decreasing interest in autochthonous goat breeds. As a result, the size of local goat populations in Bulgaria gradually decreased. In the last few decades however the interest in preserving local goat breeds, both by farmers and by researchers, has been re-appearing.

For the present study we selected two local goat breeds with a distinctive exterior, stably transmitted in the progeny – Kaloferska long-haired goat and Bulgarian Vitoroga long-haired goat (Sedefchev et al., 2011; Vuchkov and Dimov, 2011; Vuchkov et al., 2011). Both local breeds were clearly distinguished in the past by their area of distribution – Kaloferska long-haired goat was bred mostly in the foothills of the Central Stara Planina, and Bulgarian Vitoroga long-haired goat – mainly in the mountains of

Southwest Bulgaria. Nowadays "blurring" of the boundaries of the original areas of distribution of these two breeds is occurring.

Kaloferska long-haired goat and Bulgarian Vitoroga long-haired goat are available as relatively small populations. Co-existence and successful conservation breeding of both goat populations as purebred lines require a clear differentiation by both phenotype and genotype. This, in turn, means determining the genetic diversity within the two populations and the genetic distance between them is essential.

At present, genetic research on local goat populations has not been done and therefore valuable information is missing. Furthermore, advances in genetic technology have made the application of DNA-based genetic markers relatively easy and accessible. It is for the above reasons that the two local goat breeds were selected for studying their within-breed genetic variation with molecular markers.

Material and Methods

Extraction of genomic DNA

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 (Analitica-Jena). Manufacturer protocol was followed and the steps were optimized for the conditions of our lab. Visualization of the isolated genomic DNA was done after electrophoretic separation of the products in 1% agarose gel by staining with ethidium bromide. Five µl of the final solution with extracted DNA were applied to every slot.

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

Primer name	DNA sequence	Length (bp)	Calculated melting temperature (°C)
G1	AG(8)CTG	19	48.2
G2	AG(8)YT	18	51.4
G3	GA(8)YC	18	53.9
G4	CT(8)RG	18	53.9

ISSR (Inter-Simple Sequence Repeats) analysis

ISSR analyses were performed by PCR reactions in QB-96 Thermal Cycler (Quanta Biotech, London, UK). Sequences of ISSR primers used to perform PCR reactions are listed in Table 1.

PCR reactions were performed in 25 µl reaction volumes, where for each reaction PCR buffer – 2.5 µl; dNTPs – 1.5 µl; ISSR primer – 1.5 µl; Taq – 0.12 µl; H_2O – 18.38 µl; and 1 µl genomic DNA were used.

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

Statistical analysis

Total levels of polymorphism were evaluated from produced multilocus anonymous dominant markers. 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

Genomic DNA from the two goat genotypes was isolated with the standard innuPREP DNA Kit (Analitica-Jena), according to the supplier recommendations. Optimization of the isolation protocol resulted in obtaining sufficient quantity of genomic DNA with a good quality when 15 hair follicles were used (Fig. 1).

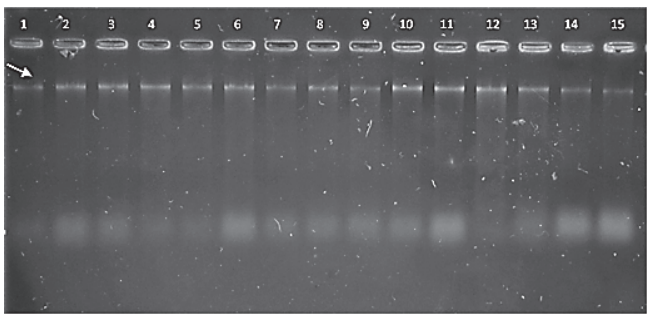

Fig. 1. Results from the extraction of genomic DNA (15 hair follicles/animal) with innuPREP DNA Kit (arrow points to the position of migrating genomic DNA)

On average 300 to 500 µg of genomic DNA were obtained as a result. When the quantity or the quality of obtained DNA was considered inadequate DNA extraction procedure was repeated till satisfactory results were obtained.

ISSR analysis

Initial screening with pre-selected ISSR markers was aimed at verifying the capacity of the selected marker system to reveal sufficient polymorphism within the two breeds. As exemplified in Fig. 2 by the products of PCR reaction with primer G1 using the selected ISSR primers produced a number of polymorphic bands between individual animals.

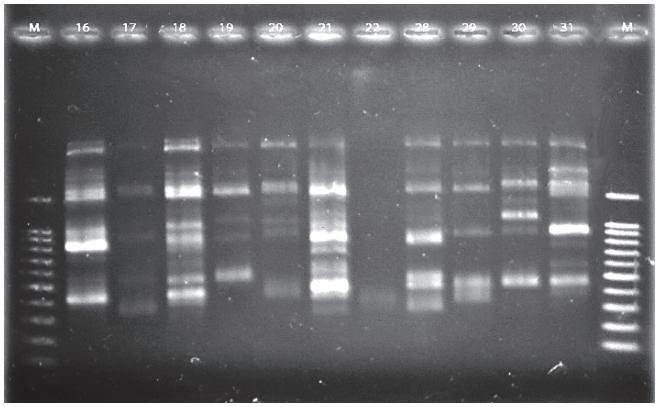

Fig. 2. Polymorphisms within Kaloferska long-haired breed as revealed after amplification with G1 primer. Lanes M – 100bp DNA ladder; Numbered lanes – PCR products from different animals

As expected the use of different ISSR primers led to revealing different numbers of polymorphic bands in the two breeds (Fig. 3). Screening several primers and optimizing the PCR conditions led to the identification of 3 ISSR primers (G1, G2 and G3) that produced informative polymorphisms in these local breeds.

The use of the selected ISSR primers resulted in generating of 18 polymorphic bands in total. This number of polymorphisms was enough to produce unique profiles for each animal of the two breeds. After the 4 ISSR primers were tested, the data was used to calculate the relative genetic distances and the animals were grouped according to them. The clustering obtained corresponded well with the known relations between individuals (Fig. 4 and Fig. 5). Within Kaloferska long-haired breed two groups of different sizes were obtained where the smaller one consisted of 16 animals (Fig. 4). Within Bulgarian Vitoroga long-haired breed on the other hand, the two main groups were much more equal in size (Fig. 5). While the smaller one consisted of 28 animals the bigger one was composed of 33 animals.

One of the basic requirements in animal breeding programs is the investigation of the existing genetic variation, whereas high genetic variation is needed for genetic improvement (Askari et al., 2011). In recent years, genetic markers are increasingly used for studying genetic diversity. Moreover, the polymorphisms determined by these markers are one of the valuable parameters for understanding their genetic differences both within and between populations.

Being highly polymorphic, microsatellites are very informative markers and have been extensively used in diversity studies. A large number of these hypervariable markers are available in goat (Seki et al., 2012). Their genotyping and scoring, however, are labor-intensive, and allele size calling

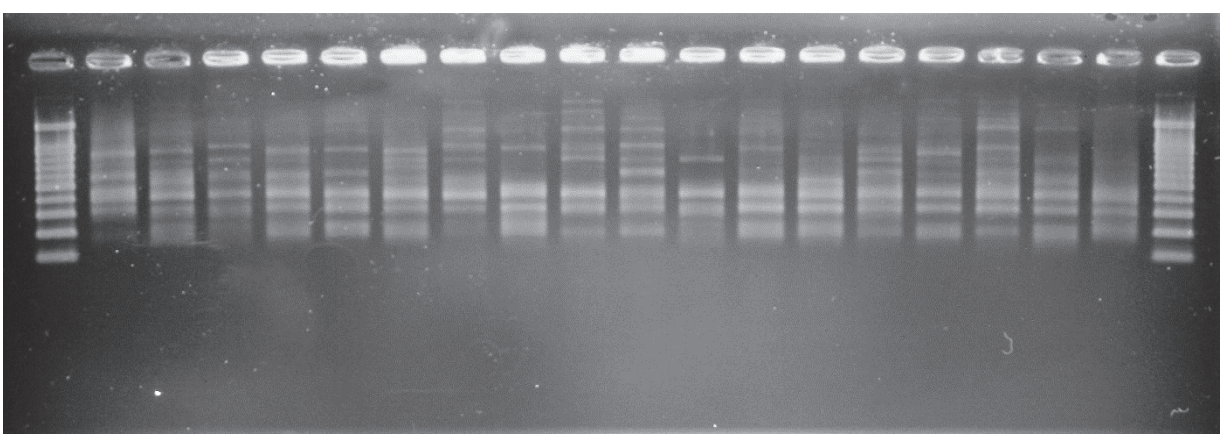

Fig. 3. Polymorphisms within Bulgarian Vitoroga long-haired breed as revealed after amplification with G2 primer. Lanes M – 100bp DNA ladder; Numbered lanes – PCR products from different animals

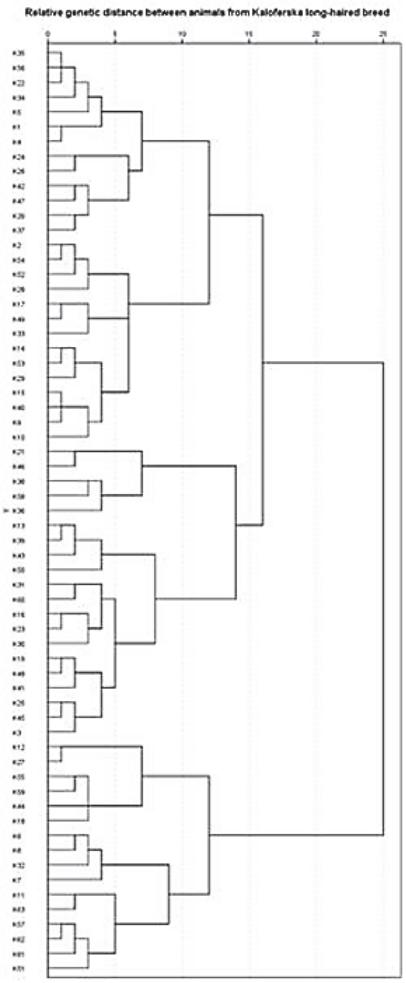

Fig. 4. Grouping of animals from Kaloferska long-haired breed based on polymorphisms revealed with G1, G2 and G3 ISSR primers

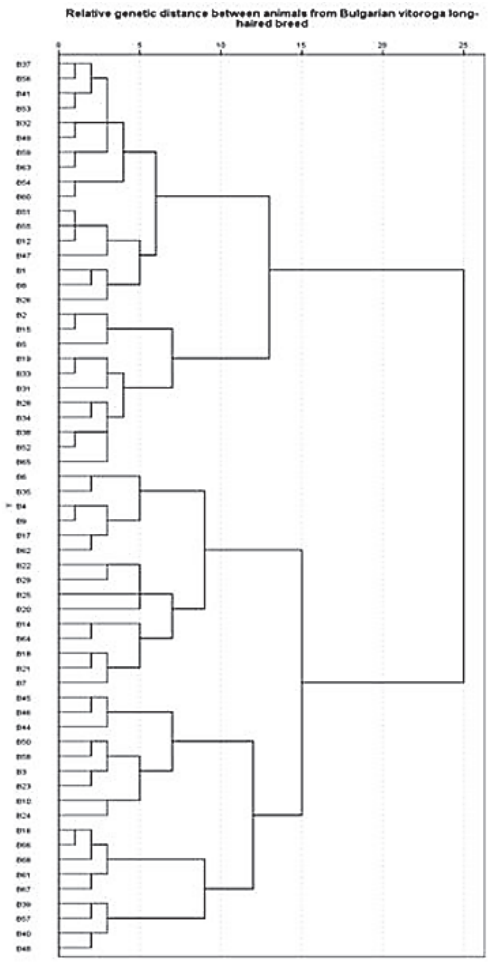

Fig. 5. Grouping of animals from Bulgarian Vitoroga long-haired breed based on polymorphisms revealed with G1, G2 and G3 ISSR primers

is difficult to be calibrated across the laboratories in the absence of a substantial number of shared samples.

The application of the ISSR marker system in our study demonstrates the potential to reveal informative polymorphisms in local long-haired goat breeds. While the use of three different primers was needed to achieve satisfactory differentiation, efficient application of the technology will require identification of the least number of PCR reactions that can produce such resolution. Expanding the number of ISSR primers in future studies should provide for such optimization.

The two dendrograms produced with the data generated within the present study (Fig. 4 and 5) demonstrate the capacity of the selected system to identify informative polymorphisms in local Bulgarian long-haired goats.

The groupings obtained for the two breeds are based on a relatively small numbers of polymorphisms. Nonetheless it appeared possible to differentiate every single animal within each of the two breeds. Therefore, the application of ISSR marker system applied within the present study demonstrates the capacity needed for producing informative polymorphisms so that satisfactory differentiation between individuals can be obtained. Furthermore, the system produced groupings of the animals within the breeds that correspond well to their known relatedness. This is an important step towards developing efficient genotyping system for individual animals within each breed so that our existing knowledge on their relatedness can be enriched and complemented.

Greater understanding of the potential of native populations is necessary for supporting long-term preservation and the potential for their genetic improvement. The results from the present study suggest that the selected marker system (ISSR) is efficient in discriminating genotypes at the molecular level. This supports its future use for genetic diversity analysis for domestic goat breeds in Bulgaria both for preservation and breed improvement purposes.

Conclusions

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

Conducted molecular analyzes demonstrated that the ISSR marker system is capable of identifying genetic diversity within both local autochthonous breeds.

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Received: March 9; *Accepted*: April 19; *Published*: December 31