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Phenotypic Characterization of the Zebu Cattle in Tajikistan

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Abstract—This article deals with the genetic characteristics of samples Schwyz-zebu cattle from three farms of the Republic of Tajikistan on 10 microsatellite markers (STS). Hence, the present study was carried out to evaluate the heterozygosity in the population and to characterize this breed by identifying DNA markers using microstatellites. Microsatellites often have multiple alleles and may have heterozygosity frequencies of 70% or more. This makes them highly informative for genetic analysis. A total of ten microsatellite primers were used for microsatellite analysis in genomic DNA of Zebu cattle. The amplified products were analysed for polymorphic alleles and their frequencies. The resulting information can be used in dealing with the conservation and sustainable use of genetic resources of the Tajik Schwyz-zebu cattle.

Keywords—DNA, gene pool, Schwyz-zebu cattle, microsatellite loci.

I. Introduction

THE domestication of livestock species and a long history of migrations, selection and adaptation have created an enormous variety of breeds. Conservation of these genetic resources relies on demographic characterization, recording of production environments and effective data management. In addition, molecular genetic studies allow a comparison of genetic diversity within and across breeds and a reconstruction of the history of breeds and ancestral populations. This has been summarized for cattle, yak, water buffalo, sheep, goats, camelids, pigs, horses, and chickens. Further progress is expected to benefit from advances in molecular technology. The livestock genetic diversity is endangered as evidenced by the critic situation of more than 30% of global mammalian and avian livestock breeds which represents approximately 1500 breeds [21]. The breeders' and/or consumers preferences' for certain breeds have been identified as one of the main factor responsible for the declining genetic diversity of livestock. Such focuses on some breeds lead to the disappearance of many traditional breeds and hence to the increase of livestock products uniformity [20]. Impacts are really worrying with a loss of disease resistance and tolerance to extreme environmental conditions. The need to conserve and protect biological diversity on a global scale was established in the framework of the Rio Convention, adopted in 1992 [19]. Since then, the principle of maintaining biodiversity has been central to the various conventions and principles of sustainable development. Biodiversity refers to the comprehensive term for the degree of nature's variety; it is the whole of living beings, microorganisms, plants or animals. It is also the interactions that connect them to each other and to the environment in which they live. Biodiversity is therefore a much broader concept than the simple collection of animal and plant species that is often reduced: it is the diversity of life at all levels of organization, from gene to species and ecosystems. Biodiversity is represented by three major levels, namely: species diversity, ecosystem diversity and genetic diversity [1], which encompasses the diversity of genes of all living organisms. Genes are supported by DNA. They are transmitted during reproduction. Genetic diversity influences the diversity of traits, an individual, a population, or a species.

The development of cattle breeding in the Republic of Tajikistan is aimed at improving long term cattle' productivity [2], which is impossible without the improvement of breeding' methods, the conservation and rational use of the genes pool of local breeds, including combining valuable genetic potential of different species [3], [4]. In recent years, several cattle' breeding programs have been using a Schwyz-zebu to produce a number of hybrid breeds with improved meat and dairy productivity [5]. In this regard, the zebra-like (Zebuvide) cattle breed in Tajikistan is of undoubted interest for breeding programs [6].

Another essential condition for the effective conduct of breeding programs is based on the rational use of the gene pool, the control of the origin and genetic variation of the pedigree material [7], [8]. The population size of Schwyz-zebu breed is reducing because of crossbreeding programmes and mechanization of agriculture in its breeding tract. Hence, the present study was carried out to evaluate the heterozygosity in the population and to characterize this breed by identifying DNA markers using microstatellites. Microsatellites often have multiple alleles and may have heterozygosity frequencies of 70% or more. This makes them highly informative for genetic analysis. In addition, the loci are small enough to be analysed using Polymerase Chain Reaction. The efficiency of a marker depends on informativeness of a polymorphic marker. It depends upon the number of alleles and their population frequencies. Marker informativeness is more easily

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estimated by simply counting the number of heterozygotes in a suitably large sample set. Keeping the background information in mind, this study was undertaken to identify the DNA markers in Schwyz-zebu cattle and to characterize the Schwyz-zebu cattle by developed DNA markers [9], [10]. Obtaining such information is extremely important for analyzing the gene pool and assessing the degree of phylogenetic proximity of the breeds, which is a prerequisite for the conservation and rational use of animal genetic resources [11].

In our study, we analyzed the genetic variation of Schwyz-zebu, the cattle received from crossing zebuvides and Schwyz cattle.

II. Materials and Methods

For the analysis, wool samples were collected from Tajik Schwyz-zebu cattle. The wool samples were taken by plucking and stored at -20 °C. Under these conditions, samples can be stored for an unlimited period of time.

The material was obtained from three farms in the Tajikistan Republic. In total, 25 DNA samples were examined, and were grouped into 3 groups, according to their origin (farm):

- Group 1-7: Samples from the Livestock Biotechnology Center, Rudaki district
- Group 2-6: Samples from the farm "Barakat and Evan", Yavansky district
- Group 3-12: Samples from the farm "Latif Murodova" (DNA bank of the Ernst VIZH Federal Science Center).

The studies were conducted using a multi-locus system of 10 locus specific for cattle and recommended by ISAG for comparative testing [12], [13]. The marker panel for analysis included the following locus: TGLA 227, BM2113, ETH10, SPS115, TGLA122, INRA023, TGLA126, BM1818, ETH225. BM 1824.

Electrophoretic separation of DNA fragments by capillary electrophoresis was performed on an ABI 3130xl instrument. The processing of the capillary electrophoresis data was carried out by translating the lengths of the detected fragments into numerical expressions based on a comparison of their mobility with the DNA standard [14]. Data on the alleles of each animal were summarized in the Microsoft Excel spreadsheet. The obtained matrix of genotypes served as a basis for statistical processing of the results.

When conducting comparative population-genetic studies on the characteristics of fund alleles, the following parameters were calculated to characterize studied samples: Average number of alleles (Na), frequency of alleles' occurrence, number of informative alleles, number of effective alleles (Ne), number and frequency of private alleles' occurrence; analysis of the populations' distribution [15], observed (Ho) and expected (He) degree of heterozygosity; The fixation index – F_{ST} (AMOVA). Statistical data analysis was carried out by standard methods using GenAlEx (ver 6.4.1) software and MSA_WIN 4.05 [16], [17].

III. Results

The microsatellite analysis of the samples from Schwyz-zebu cattle allowed the identification of a total of 82 alleles, with the maximum allelic diversity (61 alleles) detected in animals from group 3. In the first and second groups, 52 and 41 alleles were identified, respectively.

The total (Na) and effective (Ne) number of alleles in the locus and the Shannon index (I), as well as the average number of alleles per locus in the three groups of Schwyz-zebu cattle are summarized in Table I and Fig. 1.

As illustrated in Table I, the number of alleles in groups of animals from different farms varied from 3 in ETH10 and TGLA126 locus in the first group and TGLA 227 and TGLA126 locus in the second group to 10 in TGLA 227 locus in the third group. It should be noted that in the third group, which included a new type of Schwyz-zebu cattle, both the maximum average number of alleles and number of effective alleles were found, 6,100 ± 0,706 and 4,388 ± 0,648 alleles per locus, respectively. In the first and second groups, the average number of effective alleles was 3.718 ± 0.341, 2.934 ± 0.213, respectively. The analysis of the informative Shannon index (I), which is a measure of the species diversity, shows that I is maximal in animals of the third group with an improved type of Schwyz-zebu cattle and is quite high in animals of the first group. It is 1.51 ± 0.15 and 1.41 ± 0.11, respectively.

TABLE I
Characteristics the Cattle Breed at 10 Microsatellits Locus (Number of Alleles and Shannon Index). the Cattle Breed

Groups		TGLA227	BM 2113	ETH 10	SPS 115	TGLA122	INRA 023	TGLA126	BM 1818	ETH 225	BM 1824	**Average**
	Na	6	5	3	4	9	6	3	6	6	4	5,200±0,573
Group I	Ne	4,16	3,76	2,57	2,97	5,44	4,54	2,27	4,50	4,45	2,48	3,718±0,341
	I	1,609	1,433	1,011	1,197	1,965	1,643	0,898	1,633	1,611	1,119	1,412±0,108
	Na	3	4	5	5	4	5	3	4	4	4	4,100±0,233
Group II	Ne	2,66	3,78	2,57	4,00	3,00	3,00	1,94	2,05	3,42	2,88	2,934±0,213
	I	1,040	1,358	1,234	1,474	1,242	1,314	0,824	0,983	1,309	1,199	1,198±0,062
	Na	10	8	6	4	4	4	5	8	8	4	6,100±0,706
Group III	Ne	6,698	6,857	4,881	1,823	2,142	2,547	3,408	6,857	5,760	2,909	4,388±0,648
	I	2,073	1,990	1,658	0,836	0,983	1,073	1,403	1,990	1,886	1,207	1,510±0,148

Note: light gray - the minimum value, dark gray - the maximum number of alleles

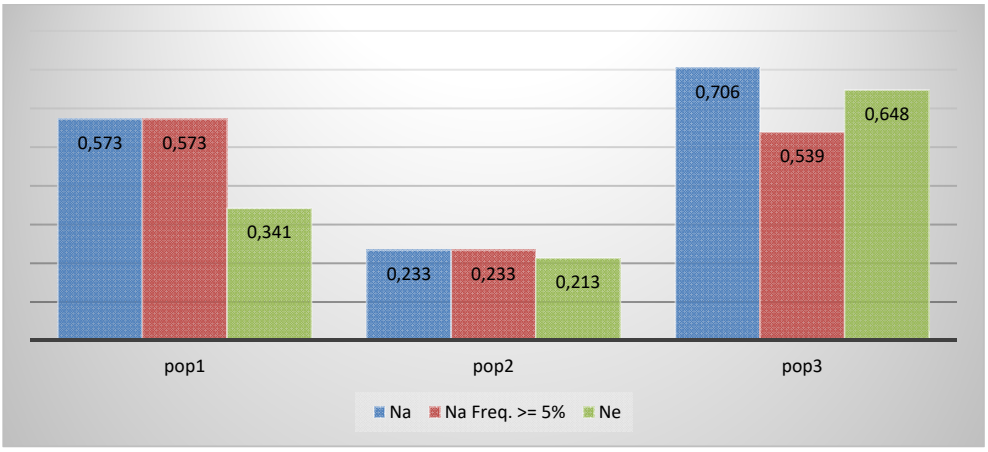

Fig. 1 Mean Allelic Patterns across Population

To estimate the level of genetic variation in the studied groups, we calculated the expected and observed degrees of heterozygosity. As known, the heterozygosity index is a reflection of mutational processes, different types of selection, gene drift, non-random mattings and other factors of population dynamics. The results of the analysis of heterozygosity levels are summarized in Table II.

TABLE II
THE ACTUAL AND EXPECTED DEGREE OF HETEROZYGOSITY OF THE ANIMALS STUDIED

Groups	Degree of heterozygosity		Difference Ho-He	F_{ST} (Fixation index)
	Actual (observed) Ho	Expected He		
Group I	0,751±0,067	0,708±0,029	0,043	-0,048±0,065
Group II	0,767±0,057	0,642±0,028	0,125	-0,187±0,072
Group III	0,695±0,084	0,714±0,046	-0,019	0,050±0,081

Data from Table II indicates that the actual degree of heterozygosity varied from 75.1% in animals from group 1 to 69.5% in animals of improved type (group 3). A small deficit of heterozygotes was detected only in group 3 – 1.9%, whereas in groups 1 and 2, an excess of heterozygous genotypes was observed from 4.3 to 12.5%, respectively. The small heterozygous deficiency in the third group is also shown by the positive value of F_{ST} – 5.0%. In the first and second groups, F_{ST} is negative. Deficiency of heterozygotes in group 3 can be explained by a probable use of moderate inbreeding for the selection of Schwyz-zebu cattle in the farm "Latif Murodova".

The calculation of the F_{ST} value revealed an excess of heterozygotes in six microsatellites: TGLA 227, SPS 115, TGLA126, ETH 225, BM 1824, TGLA122, from 4.5 to 30.5% in TGLA227 and TGLA122, respectively. And at the same time, a heterozygote deficiency was detected in four microsatellite loci from 0.2-0.9% in BM 2113 and ETH 10 to 7.2-9.9% in INRA 023 and BM1818.

The average F_{ST} value for the 10 SSR loci for all studied breeds was 0.124, which indicates that 87.6% of all variability is due to intra-breed diversity and 12.4% to inter-breed diversity.

Obtained results were used to evaluate the genetic relationship of studied samples. Our evaluation was based on the SSR analysis of the genotypes of each individual, as well as on the number and frequency of alleles common to each of the populations (Fig. 2).

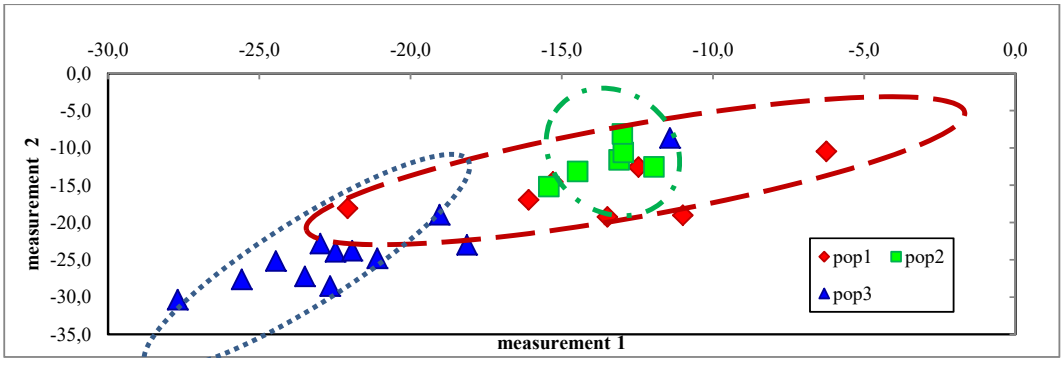

Fig. 2 A two-dimensional distribution of studied samples appurtenance to own population in the studied groups of Shvicezebuvides cattle based on SSR locus polymorphism

Belonging to the own population in the first group was the lowest among the studied groups and amounted to 71.42%, in

the second – 83.33% and in the group with improved type of Schwyz-zebu cattle – 91.67%. On average, 84.00% of the animals showed their appurtenance to their own population, while 16.00% were migrants.

As shown in Table III, from the studied groups, groups 1 and 2 are genetically close, while group 3, which included an improved type of local Schwyz-zebu cattle, is genetically more remote from them. It can be explained by a probably increased proportion of *Bos taurus* blood in the animals of this group, which led to significant changes in the allelofond of the Schwyz-zebu cattle from the farm "Latif Murodova».

Analysis of Fig. 3 showed that all studied animals of the three farms are grouped into two large clusters, the first consisting representatives of Groups 1 and 2,which unites the representatives the local zebu of livestock, while the second is represented exclusively by individuals of the improved type obtained by the inflow blood from the Schwitz breed to the local zebu cattle of Tajikistan.

TABLE III
GENETIC DISTANCES BETWEEN THE INVESTIGATED GROUPS OF ZEBU LIVESTOCK

Groups	GroupI	GroupII	GroupIII
Group1	*	0,1246	0,4210
Group2	0,2599	*	0,4874
Group3	0,3844	0,4319	*

Note: above the axis, the values obtained in the Nei calculation (the standard genetic distance corrected for a small sample size), under the axis, the frequency chord values [18].

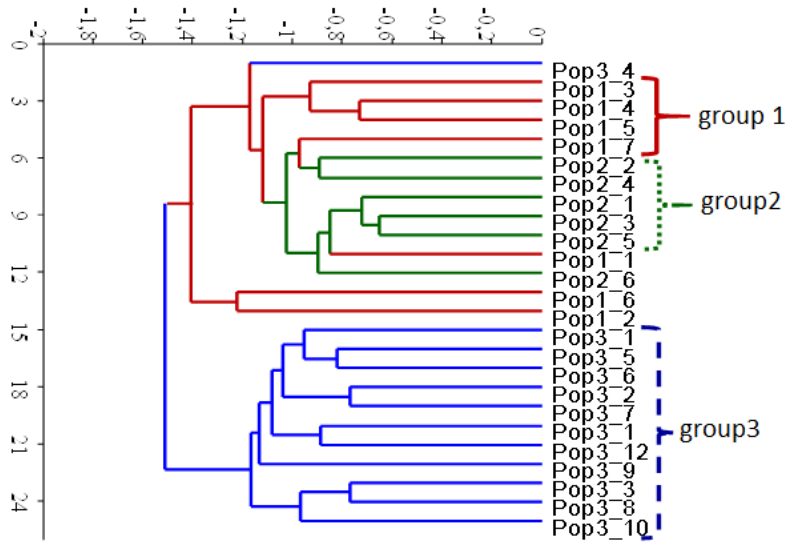

Fig. 3 Dendrogram of phylogenetic relationship of studied zebu cattle in Tajikistan

IV. CONCLUSION

The microsatellite analysis of the entire sample of Schwyz-zebu cattle we identified 82 alleles in total, with the highest number of alleles – 61 alleles found in animals of the 3rd group. In Groups 1 and 2, 52 and 41 alleles were identified, respectively.

The maximum average number of effective alleles – 4.388 ± 0.648 was also in the third group. In the first and second groups, the average number of effective alleles was 3.718 ± 0.341 and 2.934 ± 0.213, respectively.

The improved type of local Schwyz-zebu cattle in Tajikistan was the most genetically distant from animals from the first and second groups, which is probably due to an increased proportion of *Bos taurus* blood in the allelefond of the third group' animals.

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