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Genetic Diversity of the N'Dama Breed in Mali Using SSR Markers

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Abstract: The N'Dama race, trypanotolerant and well adapted to the climatic conditions of Mali, is threatened of disappearance by the introduction of other genes by means of the artificial insemination with the exotic races or zebus. In order to adopt preservation and conservation strategies, it is important to study the genetic characteristics of the race across the country. In this study, carried out on the race in its cradle (Yanfolila district, Sikasso region), the genetic diversity of 119 N'Dama race, from the Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock, was evaluated with 9 microsatellite markers (SSR). A total of 60 alleles were obtained. The number of alleles varied from 2 (BM 1824) to 12 (INRA 37) with an average of 6.67 per locus. The PIC ranked from 0.39 (BM 1824) to 0.9183 (INRA 37) with an average of 0.6605. Genetic diversity ranged from 0.4293 (BM 1824) to 0.9228 (INRA) with an average of 0.6908. The 119 N'Dama races were classified into two groups I and J according to the genetic similarities revealed by the 9 SSR markers using the UPGMA method. Group J was formed with the majority (85%) of individuals and composed of four (4) clusters J_1, J_2, J_3 and J_4. The 69 Nd, 71 Nd and 72 Nd individuals showed strong dissimilar compared to other individuals in group J and formed cluster J_1. Moreover, one cluster, with 15% of individuals, was belonged to Group I. The results of this study will contribute to the application of molecular tools and strengthen strategies for conservation, preservation and genetic improvement of the N'Dama race in Mali.

Keywords: Genetic Diversity, N'Dama, SSR Marker, Mali

1. Introduction

N'Dama is a rustic breed and well adapted to the southern humid environment of Mali [1]. Beyond its trypanotolerant nature and resistance to high humidity, this breed is more tolerant to hunger and thirst. N'Dama oxen are well adapted to hitching up in agriculture and less demanding in terms of health care [1]. These characters make it more interesting with most of the livestock householders and those in charge of animal product development [2]. With the increasing demand for animal products (milk and meat), breeders tend to introduce other genes through artificial insemination with exotic breeds or by simple interbreeding with zebus due to increasing demand for animal products (milk and meat) [1; 3]. This situation contributes to miscegenation of the N'Dama and can lead to a loss of genes of interest with decreasing of N'Dama number in the environment since crosses are more sensitive to environmental factors with expensive health care [1]. Therefore, genetic diversity study will be one of the strategies management N'Dama herd for conservation and also select the best genotypes for breeding program. Several genetic diversity studies have been conducted on cattle breeds across the world [4-7]. In Africa, Goudarzi *et al.* [7] studied genetic variability of 201 individuals of the Somba cattle race from Benin and Togo with 33 microsatellite markers whereas, Gororo *et al.* [28] study's was about 50

Sanga cattle race from Zimbabwe using 16 SSRs. In Senegal, N'diaye *et al.* [5] evaluated the genetic diversity of 120 cattle composed of four races from Senegal including N'Dama race using 12 SSR markers. In Mali, there is little information on the genetic diversity of our indigenous races, especially N'Dama. Simple sequence repeats (SSR) markers are commonly used in molecular genetic studies such as genetic linkage mapping [8] and population structures [9] due to their reproducibility, polymorphism and dominance [10; 11]. Thirty SSR markers have been recommended by the International Society of Animal Genetics (ISAG) and the Food and Agriculture Organization (FAO) for the genetic characterization of cattle [12]. In this study, we evaluated the genetic diversity of 119 individuals of N'Dama in Madina Diassa ranch using 9 microsatellite markers per PCR.

2. Material and Methods

2.1. Herd Management

The herd of Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock at Yanfolila in the Sikasso region was composed of two batches of 241 individuals in total. The first batch, was adapted by ONDY (Operation N'Dama Yanfolila) and the second was introduced few years later by PROGEBE (Regional Project for Sustainable Management of Endemic Ruminant Livestock in West Africa) respectively in Yanfolila, Kita and Kenieba environments. The animals were split up to two separated parks with two male parents each. All animals were kept together except calves less than 3 months old. The mating monitoring was done by technicians and shepherds of the Center. Sometimes, undesirables mating were done by young male which have not been kept in the herd as male parents. Most of the animals in the center have been purchased and had a very homogeneous shade color with dominant tawny.

2.2. Blood Sample Collection

Sampling was done in April 2015 on the Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock. Four milliliters (4 ml) of blood were collected in EDTA tubes from 119 individuals randomly selected. Samples were tagged, stored in ice and sent to the Laboratory of Microbiology and Microbial Biotechnology Research (LaboREM-Biotech) of the Faculty of Science and Technology (FST) for molecular analysis.

2.3. Extraction of Genomic DNA

Genomic DNA of N'Dama was extracted from whole blood with the Promega ReliaPrepTM Blood Extraction Kit gDNA Miniprep System. The concentration of the DNA was determined using the Eppendorf Spectrophotometer. DNA samples were diluted into 20 ng/µl with Water Nuclease Free VWR and stored at -20°C.

2.4. DNA Amplification PCR

DNA samples were amplified with 9 primer pairs of SSR markers (Table 1). The PCR was perfomed using a reactive mixture of 25 µl composed of Promega PCR kit ingredient (8.5 µl of pure water, 12.5 µl of Go Taq Green Master Mix 2X, 1 µl of forward primer 100 pmol and 1 µl of 100 pmol reverse primer) and 2 µl of 20 ng/µl genomic DNA. The mixture was partitioned between PCR 8-Strip tubes with Strip. Amplification was performed with the thermocycler TECHNE-PRIME according to the following program: initial denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, hybridization varying between 51-57°C, elongation at 72°C for 1 mn 45s, final elongation at 72°C for 10 min and storage at 4°C. Denaturation, hybridization, and elongation constituted one cycle repeated at 35 times.

Table 1. *Sequences and Repetitive Patterns of SSR Primers.*

Name	Chrs		Primers	References
ILSTS005	10	Forward Reverse	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	[13]
INRA063	18	Forward Reverse	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	[14]
MM12	9	Forward Reverse	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	[15]
BM1824	1	Forward Reverse	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	[16]
ILSTS011	14	Forward Reverse	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	[13]
TGLA 122	21	Forward Reverse	CCCTCCTCCAGGTAAATC AGC AATCACATGGCAAATAAGTACATAC	[17]
TGLA 53	16	Forward Reverse	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	[17]
INRA37	11	Forward Reverse	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	[14]
INRA172	26	Forward Reverse	CCACTTCCCTGTATCCTCCTCCT GGTGCTCCCATTGTGTAGAC	[18]

Chrs: chromosome

2.5. Electrophoresis of Amplified Products

PCR product was loaded into 3% (w / v) agarose MS-4 gel, prepared with TBE 0.5X (Tris, EDTA Acid Borate) and 30 µl of 10% ethidium bromide (1 mg/ml) [19]. The gel was running for 2 hours 30 minutes at 80V and photographed with Gel Documentation System E-BOX VX2 version 15.06.

2.6. Statistical Analyzes

Allele size of each SSR marker was determined in base pairs using the E-Capt software version 15.06. The diversity of N'Dama was assessed based on the number of alleles, frequency of alleles, genetic diversity and the PIC (Polymorphism Information Content). These statistical parameters were calculated according to Dao *et al*. [19]. The matrix of genetic distance was determined using Power Marker software version 3.25 [20]. The phylogenetic tree was constructed using UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) and edited with MEGA 7.0 [19; 21].

3. Results and Discussion

Molecular characterization showed a strong allelic variability of N'Dama revealing a total of 60 alleles. The number of allele ranged from 2 (BM 1824) to 12 (INRA 37) with an average of 6.67 per locus (Table 2). The highest allele frequency was observed at the locus BM 1824 on chromosome 1 and the lowest at the locus INRA37 on chromosome 11. The PIC ranged from 0.39 (BM 1824) to 0.9183 (INRA 37) with an average of 0.6605. All SSRs had a PIC greater than 0.50 except ILSTS005 and BM1824. The genetic diversity is proportional to PIC, in this study it was ranged from 0.4293 to 0.9228 with an average of 0.6908 for the same SSRs.

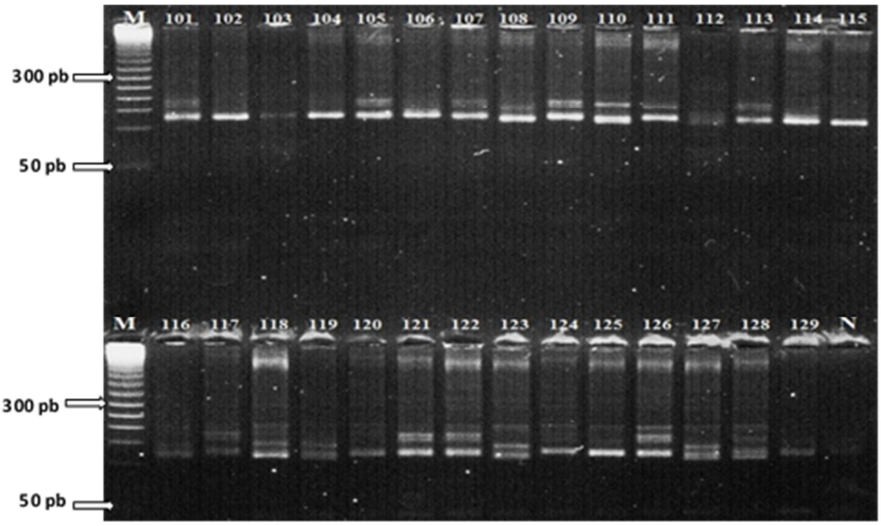

Figure 1. *PCR product profils from N'Dama with INRA37 SSR makers on Agarose MS-4 gel. M: size marker (in base pair), N: Negative control, 161 to 129: samples N ' Damas.*

Table 2. *Genetic diversity and polymorphism information content of N'Dama in Madina Diassa Ranch.*

		allele size (pb)					
Marker	**Number of alleles**	**Max**	**Min**	**Allele No**	**Major allele frequency**	**Gene diversity**	**PIC**
BM 1824	2	223	178	3	0.7311	0.4293	0.39
MM 12	7	443	59	9	0.5882	0.6077	0.5744
TGLA 53	7	241	138	19	0.3949	0.8071	0.7946
ILSTS 011	7	561	80	8	0.2689	0.7925	0.7612
TGLA 122	6	380	144	6	0.3697	0.6805	0.6167
INRA 172	10	300	100	16	0.3361	0.7916	0.765
INRA 063	6	450	103	9	0.5126	0.683	0.6526
INRA 37	12	245	61	33	0.1681	0.9228	0.9183
ILSTS 005	3	232	67	5	0.6807	0.5031	0.4712
Mean	6.67					0.6908	0.6605

PIC: Polymorphism Information Content

Referring to the formula of Botstein *et al*. [22], 98% of SSR markers were highly informative with a PIC greater than 0.5. A similar result was obtained by Kumar *et al*. [23] with 95% of SSRs on the Hallikar breed in India. Kramarenko *et al*. [24] also reported that all the loci of the Red cattle breed population were highly polymorphic. The genetic diversity of this study is specific to the N'Dama breed of Madina-Diassa Center for Preservation, Multiplication and Dissemination of Endemic Ruminant Livestock and depends on the breeding environments. However the same value of PIC was observed by Ndiaye *et al*. [5] on the N'Dama of Kolda (Eastern Senegal and Upper Casamance). According to Grema *et al*.

[25] the level of genetic diversity may be due to historical breed mixture and current breeding practices in the region. The Madina Diassa ranch N'Dama is considered as pure, the genetic information revealed in current study may be used as basic information for conservation and diversity monitoring of the breed. The average PIC observed in this study was lower than that of Barani *et al*. [26] and Hussain *et al*. [4] who worked respectively on 50 unrelated Pulikulam cattle and 11 Pakistani breeds. Similarly, Chaudhari *et al.* [27] obtained an average PIC values of 0.59 and 0.65 for genetic diversity of 45 individuals of Kenkatha and Gaolao breeds using 25 SSRs. These average values of PICs were close to those of current study where only five SSRs were used in common (MM12, INRA63, ILSTS011, ILSTS005, and BM1824). Furthermore, Gororo *et al*. [28] found PIC values close to those of actual study with TGLA53 and TGLA 122 using 16 SSR on three breeds from Zimbabwe. The PIC value of INRA37 reported by Harton and Angus [29] on two breeds was lower than that of current founding. Genetic diversity analysis of 20 Ongole cattle showed PIC of TGLA 122 and INRA063 similar to those of the present study [30]. The total number of allele observed in this study was lower than that of Lirón *et al*. [11] and Moazami-Goudarzi *et al*. [31], who worked respectively on 10 breeds (4 Creole cattle from Argentina and Bolivia, 4 European bull breeds and 2 American zebu populations) and 11 French cattle breeds. Similarly, 142 alleles were obtained by Montoya *et al*. [29] who characterized five breeds of Colombia with 10 SSR markers. Joshi *et al*. [32] obtained only two alleles with INRA063 from 30 unrelated Nagori cattle. These differences were related to the size of the samples, the breeds as well as the different targeted loci. Teneva *et al*. [33] observed fragments of similar size on 35 Bulgarian cattle with TGLA53, TGLA122 and BM1824. The present study showed different distribution of allelic frequencies at the same loci of different breeds. The finding informations may be used for strategies adoption against introduction of foreign genes [31; 34].

Study classified the 119 N'Dama into two major Groups I and J with high variability within the groups (Figure 2). More than 80% of study materials was under Group J and shared between four (4) clusters (J_1, J_2, J_3 and J_4). Clusters J_3 and J_4 were composed of 31% and 32% individuals, respectively. Individuals in the same cluster showed strong allelic similarity to each other. Moreover, Group I consisted of one cluster had 15% of the genotypes. Individuals 69 Nd, 71 Nd and 72 Nd different to the whole genotype were belonged to cluster J_1 of Group J.

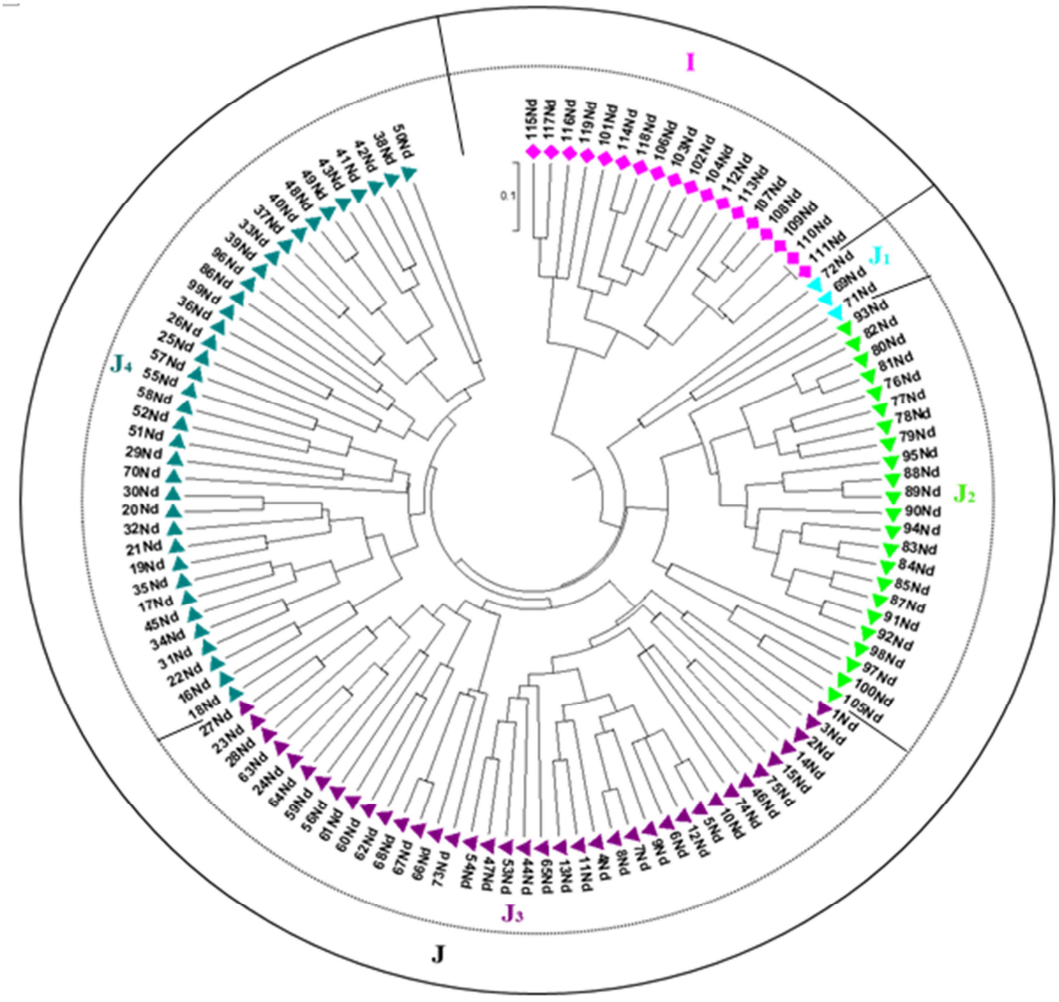

Figure 2. *Dendrogram generated from 9 SSR markers amplified 119 N'Dama genotypes based on UPGMA (unweighted pair-group method with averages).*

4. Conclusion

Molecular characterization of N'Dama race from Madina Diassa Center is important for preservation, conservation and genetic improvement strategies of cattles. This study on N'Dama race revealed high genetic diversity at the nine (9) target loci. This diversity could be related to the adaptation of the race to the living conditions into the environment and to the interconnection of individuals from different localities. Individuals 69 Nd, 71 Nd and 72 Nd showed high dissimilar and need to be followed up in the field. These data provide basic information to researchers in animal breeding field to follow up the purity of animals' race through marker-assisted selection.

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