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Genetic polymorphism study of the Tunisian Barbarine breed

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Abstract. Tunisia is well endowed with a wealth of native sheep breeds. The Barbarine, also called "Nejdi"or "Arbi", remains the main Tunisian sheep breed and it is raised in all regions with an abundance in the central region (43.3%). Although this breed has benefited from a programme of monitoring of performances for quite a long time, its productivity remains low. Thus its genetic management requires more attention. In this context the objectives of this study were to determine the genetic polymorphism in the Barbarine breed of the central region of Tunisia and to calculate genetic distances between genotyped animals. The genomic DNA of 24 purebreds Barbarine individual was extracted. These animals belong to the Tunisian Public Lands's Office (OTD) herds which are located in the governorates of Kairouan and Sidi Bouzid (centre of Tunisia). These samples were amplified by the PCR using four microsatellite molecular markers. Genotyping analysis was performed by Bio-Capt, NTSYSpc and GENAlex6.2 softwares. The value of the PIC was 0.92, showing the effectiveness of the use of the microsatellite markers in the diversity analysis. The genetic diversity's study has detected an average number of 11 alleles per locus. The allele frequencies were close and all loci were in disequilibrium compared to the act of Hardy-Weinberg equilibrium. The values of the observed heterozygosis (Ho) and the expected heterozygosis (He) were 0.23 and 0.88 respectively. Based on multi loci, the unbiased heterozygosis was 0.86 and was greater than the observed heterozygosis (0.12). The fixation index found, which reflects the deviation of the observed heterozygosity and the expected one, was positive in the range of 0.85 explaining an heterozygosis deficiency in the studied population. This means that the structure of these two farms showed an increased homogeneity between herds and management of genealogies in the mating strategy seems to be missing in the Tunisian central region, which could lead to a loss of genes.

Keywords. Sheep – Diversity– Genotyping – Polymorphism.

Etude du polymorphisme génétique au sein de la race Barbarine de Tunisie

Résumé. *La race Barbarine, appelée « Nejdi » ou « Arabi », demeure la principale race ovine tunisienne et colonise toutes les régions avec une abondance dans la région centrale (43.3%). Bien que cette race ait bénéficié d'un programme de contrôle de performances, sa productivité reste faible et sa gestion génétique nécessite encore plus d'intérêt. Les objectifs de cette étude sont de déterminer le polymorphisme génétique au sein de la race Barbarine de la région centrale de la Tunisie et de calculer les distances génétiques entre les animaux génotypés. Les ADN génomiques de 24 animaux de race Barbarine, appartenant aux troupeaux de l'OTD localisés dans les gouvernorats de Kairouan et de Sidi Bouzid, sont extraits. Ces échantillons sont amplifiés par 4 marqueurs moléculaires de type microsatellites. Le génotypage est réalisé par deux logiciels statistiques (Bio-Capt et NTSYSpc) pour la construction de dendrogramme et de la matrice de similarité entre les animaux. L'étude de la diversité génétique est faite par le logiciel GENAlex6.2. L'étude de la diversité génétique a permis de détecter un nombre moyen d'allèles par locus de 11. Les valeurs de l'hétérozygotie observée (Ho) et de l'hétérozygotie attendue (He) sont respectivement de 0,23 et 0,88. Sur la base de multi loci, l'hétérozygotie non biaisée est de 0,86 et dépasse la valeur de l'hétérozygotie observée (0,12). L'indice de fixation (F), qui traduit l'écart entre les individus trouvé à l'état hétérozygotie et l'état d'hétérozygotie attendu, est positif de l'ordre de 0,85. Ces résultats expliquent un déficit en hétérozygotie dans la population étudiée. La valeur moyenne du PIC est de 0,92 montrant l'efficacité de l'utilisation des marqueurs microsatellites dans l'analyse de la diversité. Le coefficient de similarité est très important de 0,8 soit une distance génétique très faible de 0,2. La structuration de ces deux fermes révèle une homogénéité entre les troupeaux et la gestion de la généalogie dans les stratégies d'accouplement semble être manquante dans la région centrale de la Tunisie, ce qui pourrait entrainer une perte de gènes.*

Mots-clés. *Ovin – Diversité – Génotypage – Polymorphysme.*

I – Introduction

Biodiversity defines life on earth and provides human beings with the most important services, primarily by ensuring the sustainability of vegetable production systems, and therefore by maintaining populations in their area of origin (Djemali *et al.,* 1995). Livestock contributes 30% to 40% to overall agricultural production worldwide. Among the most important genetic livestock resources, the sheep population that counts over 1064 million head, of which 18% are localized in the Mediterranean region (FAO, 2004). Tunisia is a rich country in animal resources, especially its large sheep industry, which represents an important diversity that is worth preserving and managing rationally. Among the four major sheep breeds identified in Tunisia, Barbarine remains the main breed perfectly adapted to the harsh conditions and is able to take advantage of poor land and to withstand summer heat; thereby it is raised in very different natural environments that reflect the country climatic diversity. Nevertheless Barbarine is more abundant in the Tunisian semi-arid zone, that corresponds to the central area, where it acounts for 43.3%, compared to 38% in the north and only 18.7% in the south (OEP, 2010). Unfortunately Tunisian Barbarine breed is suffering nowadays and undergoing significant diversity threat (Ben Gara, 2000; Bedhiaf-Romdhani, 2006). In this context, this work was conducted to calculate genetic distances between genotyped animals and study the genetic polymorphism in the Babarine breed of the Tunisian central region.

II – Materials and methods

Twenty-four blood samples were collected in EDTA tubes from different sheep herds that belong to the Office of State Lands (OTD) farms located in the governorates of Kairouan and Sidi Bouzid, in the Tunisian Semi-Arid Central West. Each sample contained 5 ml of blood stored at -20°C. Genomic DNA was extracted according to the standard saline protocol (Sambrook *et al.,* 1989), and was stored at -20°C in TE solution (Tris-EDTA). The DNA purity was appreciated by using the spectrophotometer. DNA quality was further verified by electrophoresis on agarose gel (0.8%) that contains 1µg/ml of Ethidium Bromide (Sambrook *et al.,* 1989), after that diluted to 50ng/µl in 100µl.

Four molecular markers *Ovis Aries* microsatellite were used to study the breed genetic diversity namely: BM1824, DYMS1, HUJ616 and ILSTS11. For each primer, PCR reaction was optimized using different concentration of MgCl2 (1.5, 2, 2.5 and 3 mM) at a temperature gradient ranged from 50 to 65°C. PCR amplification has been developed in a thermocycler (C1000, BioRad). The PCR products were migrated by electrophoresis on agarose gel (2%) for 60 mn under 90 volts with a 100bp ladder (Promega, USA), then the electrophoretic profile were reveled under UV by using a transilluminator. Estimation of genetic diversity was carried out through two statistical softwares GENALEX6.2 and NTSYS 2.0e. Six parameters were calculated to describe the population genetic variability namely Average number of allele per locus, Allele frequencies, Rate of the observed heterozygosity (Ho), Rate of the expected heterozygosity (He), Rate of the unbiased heterozygosity (HNB), Fixed index (F) and Level of polymorphism (PIC).

III – Results and discussion

DNA concentrations varied between 42.11 g/ml and 1020 g/ml. A diluted DNA was fixed to 50ng/µl for all samples.

The optimization step has allowed to determine the PCR parameters for each primer while viewing the electrophoretic profiles of the amplified products (Table 1).

After optimization, genomic DNA was amplified using the four primers according to their appropriate parameters. The genotyped product was viewed under UV (Figs 1, 2, 3 and 4).

Table 1. The PCR parameter for the primers

Locus	Range	[$MgCl_2$]mM	Temperature°C
BM1824	171-194	2,5	59.5
DYMS1	152-221	2	53
HUJ616	114-178	2,5	59.5
ILSTS11	256-300	2,5	62.5

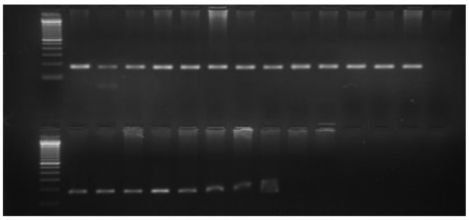

Fig. 1. Amplified DNA using BM1824.

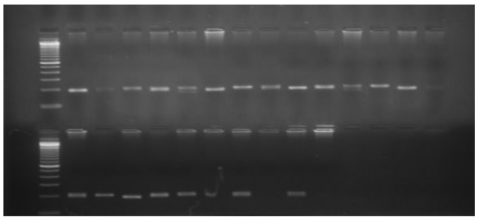

Fig. 2. Amplified DNA using DYMS1.

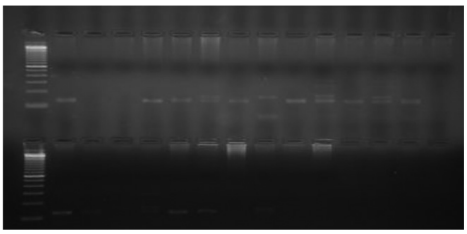

Fig. 3. Amplified DNA using HUJ616.

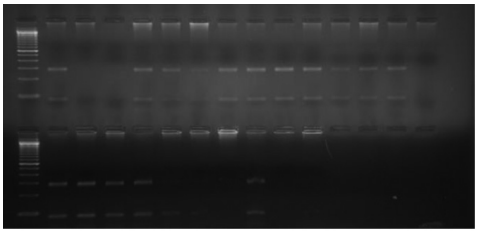

Fig. 4. Amplified DNA using ILSTS11.

Profiles were processed by BioCapt software (BioCapt, version 12.3.00, vilbert lourmat) in order to calculate the generated bands size and to construct a binary matrix. NTSYS2.0e software was used to transform the binary matrix into a similarity matrix and to schematise also the sampled individual dendrogram. The similarity matrix analysis has demonstrated high coefficients that varied between 0.78 and 0.97. The dendrogram showed two distinct groups with 0.2 genetic distances. The first group included 22 animals, whose similarity coefficients were very close and ranged between 0.81 and 0.87 suggesting that only 8% of differences were observed between the individuals. This explains a strong genetic relationship between animals although samples were taken from different farms located on two remote governorates. The second group was constructed by only one individual.

For all the studied loci, the genetic diversity analysis has revealed an average observed allele number (Ho) of about 10, 12, 14 and 7 for the locus BM1824, DYMS1, HUJ616 and ILSTS11 respectively; that exceeded the average expected allele number in the balanced population (He), in the order of 7.11, 9.13, 10.70 and 4.92 respectively. Furthermore, the fixed indexes were very high and varied between 0.77, 0.89, 0.74 and 1 for BM1824, DYMS1, HUJ616 and ILSTS11 respectively with an average of 0.85. These results indicate that the studied population is deviated from the panmictic law, whence it is in disequilibrium compared to the act of Hardy-Weinberg equilibrium.

The allele number generated per locus was about 21, 22, 17 and 16 for BM1824, DYMS1, HUJ616 and ILSTS11 respectively. Allele sizes ranged between 173 pb and 219 pb, 186 pb and 239 pb,

118 pb and 165 pb and 268 pb and 322 pb. Allele frequencies varied from 0.04 to 0.26, from 0.02 to 0.15, from 0.02 to 0.17 and from 0.06 to 0.25 respectively. The allele frequencies were very close and mostly similar for all the studied loci. Besides, the average observed heterozygosity (Ho) were remarkably exceeded by the average expected heterozygosity (He) explaining a deficit of heterozygosity and reflecting a high inbreeding rate (Table 2). For ILSTS11 locus zero was obtained meaning that all individuals are homozygous for this marker.

Table 2. Heterozygosity rates

Locus	(Ho)	(He)	Unbiased H
BBM1824	0.19	0.85	0.88
DYMS1	0.09	0.89	0.91
HUJ616	0.23	0.90	0.93
ILSTS11	0	0.79	0.82
Means	0.12	0.88	0.86
Standard deviation	0.05	0.02	0.02

The average PIC (Polymorphic Information Content) was 0.92 proving that microsatellites are effective to study the genetic diversity. The most polymorphic microsatellite were ILSTS11 (PIC = 0.97), followed by DYMS1 (PIC = 0.95), HUJ616 (PIC = 0.94) and finally BM1824 (PIC = 0.85).

IV – Conclusions

The results of this study provide a molecular characterization of a Barbarine breed sample from the Central Region of Tunisia and gave a rough idea about the breed genetic diversity in this region. Considering the abundance of this breed in the central area, the ultimate similarity found reflects that herds belonging to the OTD farms seem to be threatened by a lack of genealogies management and an increased rate of inbreeding that is ultimately due to mating between ascendants and descendants. Such a process may alter gene frequencies that may lead to genetic variability loss in governmental stations over the generations and even a decreasing in animal performance and hence the biodiversity of this breed across the country will be questioned. Thus, for sustainable management of this breed, it is recommended to perform selection and to use the most genetically distant animals in mating strategies in order to reduce inbreeding. However, because of the sample size which is relatively small and not representative enough, it may be assumed that the results found may be explained by the fact that samples are from only two governmental stations. Moreover, this study opens a door for a biodiversity study of the breed across the whole country with animals from different geographical areas.

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