An Investigation on DNA Polymorphism of the Cattle Breeds in the Province of Kars by RAPD-PCR Technique

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SUMMARY

A genetic analysis using RAPD (Random Amplified Polymorphic DNA) markers was performed to determine the breed-specific primers and designate the RAPD fingerprints and genetic diversities of cattle breeds (East Anatolian Red, Zavot, Turkish Brown Swiss and Simmental) in Kars, an important province for cattle rearing of Turkey. The DNA samples were isolated from a total of 91 animals from four breeds and eighteen random primers were screened. Genetic relations between breeds were determined by RAPD polymorphisms obtained from a total of 89 loci. Statistical analysis of the data, estimating the genetic distances between breeds and sketching the cluster trees by UPGMA method were performed with a computer program. Estimation of genetic relationships between the breeds revealed two clearly distinct groups of breeds: one consisted of East Anatolian Red and Simmental breeds, and the other included Turkish Brown Swiss and Zavot breeds. No breed-specific or sex-specific RAPD bands were detected. The study showed that the polymorphisms generated by RAPD-PCR enable the determination of genetic relationships and fingerprints of the cattle breeds in the province of Kars.

Keywords: Cattle - breed - fingerprinting - phylogeny - RAPD-PCR.

RÉSUMÉ

Une recherche sur le polymorphisme de l’ADN des races bovines dans la province de Kars par la technique de RAPD-PCR. Par A. K. DEVrim et N. KAYA.

Une analyse génétique utilisant des marqueurs RAPD a été réalisée afin de déterminer les amorces spécifiques, les empreintes RAPD et la diversité génétique des races bovines (Rouge Anatolien Est, Zavot, Brown Swiss et Simmental) dans Kars une province importante pour l’élevage bovin. Les échantillons d’ADN ont été isolés à partir de 91 animaux issus des quatre races et dix-huit amorce allatoires ont été examinées. Des relations génétiques entre les races ont été déterminées par l’analyse des polymorphismes par RAPD obtenus à partir d’un total de 89 loci. L’analyse statistique des données a permis d’évaluer les distances génétiques entre les races et de construire les arbre de failseau par la méthode d’UPGMA. L’évaluation des rapports génétiques entre les races a mis en évidence deux groupes de races clairement distincts : un groupe composé des races Rouge Anatolien Est et Simmental, et un deuxième groupe comprenant les races Brown Swiss et Zavot. Aucune bande RAPD spécifique de la race ou du sexe n’a été détectée. L’étude a montré que l’évaluation des polymorphismes par RAPD-PCR permet de déterminer les relations génétiques et les empreintes digitales des races bovines de la province de Kars.

Mots-clés: Bovin - race - Empreinte digitale - Phylogénie - RAPD-PCR.

Introduction

DNA-based polymorphism studies are revolutionizing genetic analyses of livestock species. The use of polymerase chain reaction (PCR), restrictive enzymes and automated short tandem repeat (STR) analysis in DNA sequencers allowed for identification of polymorphic markers, such as restriction fragment length polymorphism (RFLP), microsatellite and randomly amplified polymorphic DNA (RAPD) [4, 6, 14]. RAPD-PCR technique, described first by WILLIAMS et al. [17], is a quick and effective method for genetic differentiation of cattle breeds [9]. It is based on PCR amplification of genomic regions using short primers of arbitrary sequence. No prior knowledge of the genome under investigation is necessary to perform the reaction [2].

For probably taking origins from distinct wild cattle forms and applied various selection systems and different breeding techniques, the cattle breeds in the world exhibit a great diversity in comparison with their ancestors [12]. It is reported that there are 300 cattle breeds in the world originating from Bos taurus prigeminus, B. taurus brachyceros and their wild forms. Furthermore, the domestication and the distribution of the cattle to the other regions of the world was first realized in Anatolia in 5000-6000 BC. [15]. As Bos taurus was developed in Anatolia, Anatolian breeds are very important for evolution studies of today’s cattle breeds [1, 3].

Kars is a city in north-eastern Turkey, a transition region between Caucasians and Anatolia which has pasturages with rich plant flora. Although intensive cattle and sheep breeding is performed in Kars, very little is known about genetic diversity and population structure of the local breeds. Kars has 2.7% (300 970/11 185 000) of the Turkish cattle population [5]. Four cattle breeds; East Anatolian Red (EAR), Zavot (ZAV), Turkish Brown Swiss (TBS) and Simmental (SIM) are bred in the province of Kars.

The local and indigenous cattle breeds in the province of Kars are ZAV and EAR. The EAR breed is characterized by broad genetic diversity and scattered widely in east Anatolia. Genetic diversity of EAR results from its Simmental, Brown Swiss, German Red and Turkish Black genotypes. The number of EAR cattle is the second in Turkish indigenous breeds. ZAV is reared mostly for cheddar cheese production in Zavot and Dikme villages. It was obtained by hybridizing the local indigenous breeds by Simmental and Swiss Brown brought

from Caucasians. For genetic improvement of Turkish indigenous cattle breeds, Simmental and Brown Swiss genotypes have been imported several times from European countries since 1925. By hybridizing these breeds with Turkish indigenous cattle, today's TBS and SIM breeds came into existence [1]. The aim of this study was to determine the breed-specific primers and designate the RAPD fingerprints and genetic diversities of cattle breeds in Kars.

Materials and Methods

ANIMAL MATERIAL

A total of 91 animals from four breeds: EAR (21 animals), ZAV (25), TBS (24) and SIM (21), were used in this study. Animals were sampled from their native breeding ranches and villages regarding to the phenotypic appearances. It was intended to fix the genetic relations at the minimal level between the sampled animals. In this respect, sampling studies were performed on a wide region and data on the number of sires and dams were interrogated. Maximum two herds from each village and maximum two animals from each herd were sampled.

DNA ISOLATION

Peripheral blood samples (6-9 ml) were collected in EDTA-tubes. DNA samples were isolated from the leukocytes by commercial kit (MBI Fermantas®-Genomic DNA Purification Kit #K0512, USA) using the salting out DNA extraction method. Isolated DNA concentrations were measured spectrophotometrically (Spectramax® Plus 384, Molecular Devices, USA) and DNA samples were concentrated at 100 ng/µl prior to RAPD-PCR process.

PRIMERS AND PCR AMPLIFICATION

A total of 18 random primers (Table I) were performed singly on 10 animals per breed. All primers were synthesized by a commercial firm (Integrated DNA Technologies Inc. USA) and diluted with nuclease free water (AMRESCO® USA). Amplifications were done in nuclease free PCR tubes (AXY-GEN®, USA) using a programmable thermal cycler (Techne® Genius, UK). Each 52 µl reaction mix comprised 100 ng template DNA, 10 pmol primer, 125 µM dNTPs, 5 units Taq DNA polymerase (Fermentas®, #EP0402, USA), 1.5 mM MgCl₂ and 1 µl PCR buffer (Fermentas®, USA). Duplicate PCR reactions of each animal were carried out for 40 cycles; 1 min. at 94 °C (initial denaturation), 1 min. at 35°C (annealing), 1 min. at 72°C (extension) and 6 min. at 76°C (final extension). Amplification products were separated by agarose gel (1.5%) electrophoresis at 80 V and detected by ethidium bromide (0.5 µg/ml) staining. pUC19 DNA/MspI (HpaII) 34.34-500 bp (#SM0221) and 100 bp DNA Ladder 80-1031 bp (#SM0243) (MBI Fermentas® USA) were used as molecular size markers. RAPD fingerprints were visualized by UV illumination and documented by photography.

SCORING OF BANDS AND STATISTICAL ANALYSIS

Only distinct, prominent bands were scored. Genetic relations between breeds were determined by RAPD polymorphisms obtained from a total of 89 loci. The DNA bands were scored for their presence (1) or absence (0) in the

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>G+C%</th>
<th>Total number of scored bands</th>
<th>Polymorphic bands</th>
<th>Bands size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5’- AGC TGT CTC A -3’</td>
<td>50.0</td>
<td>7</td>
<td>7</td>
<td>500-1200</td>
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<tr>
<td>P2</td>
<td>5’- CGG CGC CGG T -3’</td>
<td>90.0</td>
<td>14</td>
<td>12</td>
<td>150-1100</td>
</tr>
<tr>
<td>P3</td>
<td>5’- CCA GGA CGC G -3’</td>
<td>80.0</td>
<td>10</td>
<td>10</td>
<td>300-1200</td>
</tr>
<tr>
<td>P4</td>
<td>5’- GGT CAC CTA C -3’</td>
<td>60.0</td>
<td>8</td>
<td>7</td>
<td>250-800</td>
</tr>
<tr>
<td>P5</td>
<td>5’- GGC TGC AGT G -3’</td>
<td>70.0</td>
<td>3</td>
<td>3</td>
<td>250-400</td>
</tr>
<tr>
<td>P6</td>
<td>5’- CTG CAG CGG T -3’</td>
<td>70.0</td>
<td>17</td>
<td>15</td>
<td>200-1200</td>
</tr>
<tr>
<td>P7</td>
<td>5’- CTC AGT CAC -3’</td>
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<td>6</td>
<td>6</td>
<td>550-1100</td>
</tr>
<tr>
<td>P8</td>
<td>5’- CGG CTA GGT -3’</td>
<td>66.7</td>
<td>11</td>
<td>10</td>
<td>300-1100</td>
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<tr>
<td>P9</td>
<td>5’- GCA TCA GGT -3’</td>
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<td>2</td>
<td>0</td>
<td>400-500</td>
</tr>
<tr>
<td>P10</td>
<td>5’- AAC AGC ACT CTC TTC AGG C -3’</td>
<td>52.6</td>
<td>11</td>
<td>8</td>
<td>200-1000</td>
</tr>
<tr>
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<td>5’- TTA TGT AAA ACG ACG GCC ACT -3’</td>
<td>42.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P12</td>
<td>5’- CCC AGG GTT -3’</td>
<td>66.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P13</td>
<td>5’- CGG GTG TGG G -3’</td>
<td>80.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>P14</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P15</td>
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<td>80.0</td>
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</table>

Table I. — Data about the primers used in the study.

RAPD profile of individuals from all 4 breeds. Statistical analysis of the data, estimating the genetic distances between breeds and sketching the cluster trees by un-weighted pair group analysis (UPGMA) were performed with the matrices of Nei coefficient [10] of similarity based on RAPD data by a computer program (POPGENE VERSION 1.31, Canada).

Results

Eight out of 18 random primers tested on the DNA samples were discarded since no clear and capable amplified bands were observed. The remaining 10 primers were appreciated according to the number and intensity of bands and used to evaluate genome variability. In order to score fingerprints, we assumed that one band corresponded to one locus. The number of bands amplified with these primers ranged from 2 to 17 and had a size range of 150 to 1200 bp. A total of 89 loci were amplified, out of these bands 78 (87.6%) were polymorphic. Figure 1 shows RAPD fingerprints of P6 that amplified 17 bands and Figure 2 shows RAPD fingerprints of P8 that amplified 11 bands.

By using the data in Table II, the dendogram showing genetic relations between breeds was constructed (Figure 3). Genetic relationships between the breeds revealed two clearly distinct groups of breeds: one consisted of EAR and SIM breeds, the other included TBS and ZAV breeds.

Discussion

Although some blood type [7] and protein polymorphism studies [11, 13] on Turkish cattle breeds were detected, no PCR-based genetic studies for comparison on these breeds have been previously performed. From this point of view, this was the first study investigating the genetic diversity and population structure of the cattle breeds in a transition region between Caucasians and Anatolia.

During sample collection, it became evident that there were a small number of Zavot cattle in three villages of Kars and that they have been hybridized in favour of TBS cattle. In the same way, the number of EAR cattle was also reduced as they were hybridized in favour of SIM and TBS. In this respect, a conservation program for EAR and ZAV breeds should be realized.

In this study eighteen random primers were studied and ten of them were utilized as they had clear RAPD band profiles. From 18 random primers, six 10-mers, three 9-mers, and a 19-mer produced clear RAPD band profiles. Although 10-mer primers are generally being used, 8-22-mer primers can be used in genetic studies [16]. In this study, intensively 10-

<table>
<thead>
<tr>
<th>Breed</th>
<th>EAR</th>
<th>SIM</th>
<th>ZAV</th>
<th>TBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAR</td>
<td>****</td>
<td>0.9333</td>
<td>0.9023</td>
<td>0.8670</td>
</tr>
<tr>
<td>SIM</td>
<td>0.0690</td>
<td>****</td>
<td>0.8556</td>
<td>0.8573</td>
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<td>ZAV</td>
<td>0.1028</td>
<td>0.1559</td>
<td>****</td>
<td>0.9093</td>
</tr>
<tr>
<td>TBS</td>
<td>0.1427</td>
<td>0.1540</td>
<td>0.0951</td>
<td>****</td>
</tr>
</tbody>
</table>

Table II. — Nei’s genetic identity (above diagonal) and genetic distance (below diagonal) values among breeds.

Figure 1. — RAPD amplification products generated from 2 animals of each breed by random primer P6. Lane L is molecular size marker. Two samples from each breed (T: Turkish Brown Swiss, Z: Zavot, S: Simmental and E: East Anatolian Red) are being represented on the photography. a and b RAPD bands on the gel were monomorphic for all animals in the study.

Figure 2. — RAPD amplification products generated with random primer P8. Lane L is molecular size marker exhibiting the bands that reduce with 100 bp intervals. Five samples from each breed (T: Turkish Brown Swiss, Z: Zavot, S: Simmental and E: East Anatolian Red) are being represented on the photography. On the gel a (approximately 600 bp) is monomorphic for all animals in the study and b (approx. 500 bp) is monomorphic for Turkish Brown Swiss, Simmental and East Anatolian Red breeds.
mer primers, but additionally longer and shorter primers were used to get various fingerprints.

Primer P9 produced only two RAPD bands in the scale of 440 bp and 460 bp and these two bands were monomorphic in all samples. As the bands produced by P9 are common in all animals it can be concluded that P9 may be specific for species or for greater zoological classes (gender, family etc.). This point needs to be clarified in the future by a comparison with other species. Furthermore primer P6 which produced 17 bands can be proposed for cattle fingerprint studies in the future accounting it’s RAPD band number.

Polymorphisms at the level of individual, breed, species or gender can be produced by RAPD-PCR but it is difficult to identify the bands. No breed- or sex-specific RAPD bands were detected in this study. Nevertheless, by mean of their monomorphic band profiles, some of the investigated primers can give the information that animals not having these monomorphic bands may not be related to these breeds. More studies should be performed on this matter.

Dendogram obtained by this study (Figure 3) revealed two clearly distinct groups of breeds: one consisted of EAR and SIM, and the other included TBS and ZAV breeds. As TBS and ZAV breeds have Swiss Brown genotype, it is an expected result for these two breeds to be in the same group. Alpan and Arpacik [1] reported that formerly EAR had been joined in other breeds, such as Turkish Black, Swiss Brown, German Red and Simmental. Researchers also report that these hybridizations became widespread by East Anatolian Animal Project since 1982. At recent years uncontrolled hybridizations are being increased and phylogenetic relationship of the EAR and SIM breeds is being closer because of regional animal cooperatives bringing SIM bulls since 1995 [8].

RAPD analysis revealed within as well as between-breed genetic variation among the cattle breeds in the study. All four breeds shared a high proportion of bands and 87.6% of the bands were polymorphic. The number varied with primer but it appears that ten selected primers can produce sufficient polymorphic markers.

Therefore from this investigation it can be concluded that RAPD markers are effective in detecting polymorphism between cattle breeds and provide a potential tool for studying the genetic variability and genetic relationships. More studies should be performed with different primers for detecting breed or sex specific RAPD bands.

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References