Evaluation of the Genetic Diversity in Iraqi Local Breeds of Goat Using Random Amplified Polymorphic DNA Markers

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Abstract

In the current study, random amplified polymorphic DNA polymerase (RAPD) was used to investigate the genetic diversity and relationship among three breeds of local goat (Black, Brown and White). Twelve individual were subjected to blood samples collection, four from each population. Extracted DNA was in adequate quantity and quality. The polymorphism varied between primers and within breeds. Seven out of sixteen RAPD primers were found to be polymorphic. The maximal number of scorable fragments was amplified by D15, while C5 and C12 primers were shared the same minimal value of amplified fragments which was 8 bands. Molecular size of amplified fragments ranged from 0.16 kbp to 4.8 kbp. Of the 69 amplified fragments, 32 fragments (46%) were polymorphic with an average of 4.57 polymorphic fragments per primer. Dendrogram derived from RAPD profiles separated the twelve individuals into four main clusters; two were consisted of single individual, whereas the other two clusters consisted of three and seven individuals, respectively. Genetic similarity was ranged from 0.61% to 0.97%. The results indicated that RAPD-PCR technique was a valuable tool in the arsenal of animal breeders and can efficiently distinguish between goat breeds.

تقييم التباعد الوراثي لسلالات محلية من الماعز العراقي باستخدام مؤشرات الدنا العشوائية متعددة الأشكال

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الخلاصة

تم في هذه الدراسة استخدام تقنية تضاعف سلسلة الدنا العشوائي متعدد الأشكال لإنزيم بلمرة الحامض النـــووي للتحري عن التنوع والصلة الوراثية بين ثلاث سلالات من الماعز المحلي (سوداء وبنية وبيضاء). جمعـــت عينـــات الدم من 12 فردا بواقع أربعة من كل مجموعه، وتم استخلاص الدنا منها بكمية ونوعية مناسبة. تباينت قيمة تعـــدد الأشكال الناتج فيما بين البادئات المستخدمة وسلالات الماعز. أظهرت سبعة من البادئات المستخدمة من مجموع ستة عشر حزماً متعددة الأشكال. أنتج البادئ D15 اكبر عدد من الحزم المسجلة، بينما تشارك البادئان C5 و C12 فـــي حيازتهما لأقل عدد من الحزم المسجلة والبالغة 8 حزم. تراوحت الأحجام الجزيئية للحزم الناتجة مـــن 0.16 كيلـــو زوج قاعدي إلى 4.8 كيلو زوج قاعدي. من بين 69 حزمة متضاعفة، كانت 32 منهـــا متعـــددة الأشـــكال (46%) وبمعدل 4.57 حزمة متغايرة لكل بادئ. قسمت نتائج التحليل التجميعي المعتمد على نتائج التكثير العشـــوائي متعـــدد الأشكال لإنزيم البلمرة الإفراد الاثنى عشر إلى أربع مجاميع رئيسة احتوت اثنتان منها على فرد واحد، بينما احتوت المجموعتين الرئيستين الأخريين على ثلاثة أفراد وسبعة أفراد، وبنفس التتابع. تراوحت نسبة التشابه الـــوراثي بـــين 0.61% إلى 0.97%. دلت نتائج استخدام تقنية تضاعف الدنا العشوائي متعدد الأشكال لإنزيم بلمرة الدنا على أنهـــا أداة قيمة بالنسبة لمربي الحيوان ويمكن أن تستخدم بكفاءة للتمييز بين سلالات الماعز.

Introduction

It is quite certain that the goat was one of the first domesticated animals in Western Asia (1). Dairy goats are not nearly as numerous as dairy cattle, but for many of developing countries of the world, they are the leading milk producers because they are well adapted to limited areas and require less specialized feed, so they used to call it "poor Man's cow". If genetic diversity is very low, none of the individuals in a population may have the characteristics needed to cope with the new environmental conditions or challenges. Such a population could be suddenly wiped out. Low amounts of genetic diversity increase the vulnerability of populations to catastrophic events such as disease outbreaks. Also, low genetic diversity may indicate high levels of inbreeding with its associated problems of expression of deleterious alleles or loss of over-dominance (2). RAPD markers were adopted as a powerful molecular fingerprinting technique, which is highly discriminative and allow distinction even between closely related genotypes. Genetic variation of goat breeds can provide reliable information for the selection of parental material and thus assist in breeding programs. Such reasons inspired many researchers like Oliveira *et al*, (3) to verify the genetic diversity between goat populations. Sixteen out of 120 decamer oligonucleotide primers were selected by the previously mentioned authors to estimate the genetic diversity within seven populations of Moxotó goat. The selected primers generated 56 polymorphic bands. The analysis of molecular variance showed that the greater part of total genetic variability (71.55%) was due to differences between individuals within populations, while 21.21% was among populations. Random amplification of polymorphic DNA analysis was carried out by using DNA samples of 14 black bengal goat and Jamuna pari goat breeds (4). Highest level of Nei's gene diversity value was of 0.49, and the mean genetic diversity among the 14 goat breeds was 0.37. The highest number of polymorphic bands was 32 bands. The pair-wise genetic distance value ranged from 0.25 to 1.00. Dendrogram based on Nei's genetic distance using UPGMA method indicated segregation of the 14 goat breeds. Within Jamuna pari goat genetic similarity is low as well as black Bengal goat. Genetic diversity in compared with the previous study was decreased as Li *et al*, (5) cited that the similarity was ranged from (0.90) to (0.74), as they analyzed the randomly amplified polymorphic DNA (RAPD) of 540 domesticated black goats (*Capra hircas*) from 9 different geographical populations. Authors added, a UPGMA dendrogram constructed from similarity coefficients showed that the two populations clustered together, and the other seven populations formed another group. To assess the genetic variability among six Iranian goat breeds, Esmaeelkhanian *et al* (6) used 16 arbitrary primers. Ten of these primers revealed a pattern with scorable bands. From a total number of 115 scored bands 62 (53.9%) and 53 (46.1%) were described as polymorphic and monomorphic, respectively. Nei's genetic distances varied between 0.081 and 0.227 in the population. The phylogenetic tree was reconstructed and showed two main separated groups. Another study reported that twenty seven polymorphic primers have been used to amplify the genome DNA of 15 Qianbei-Pockmarked goats, and 253 bands were detected, of which 141 were polymorphic with a polymorphism frequency range of 30%~75%, averaging 55.73%. Each primer amplified 2~14 bands, averaging 9.37. The fragment length of the amplified products ranged between 210 and 2 700 bp. Calculated by Nei formulae, the genetic similarity index ranged from 0.729 to 0.937, averaging 0.863 (7). The results of other study conducted by Yadav and Yadav (8) were within the same range, as they investigated the genetic diversity within six breed of Indian goat. The polymorphism found within breeds varied between primers and breeds. The authors added that only small proportion (40.93) of total bands amplified from 10 selected primers were polymorphic. Seven RAPD primers were selected by El Gaali and Satti (9) to study the genetic variations among 14 individuals of goat (*Capra hircus*) from two domestic Sudanese goat breeds. The test generated 59 entirely repeatable RAPD fragment bands and the statistical analysis showed 55 polymorphic bands among the 14 individuals. The genetic distances among the population

range from 8% to 72%. Authors cleared that the constructed UPGMA dendrogram showed that the individuals of the first breed were clustered together, while the individuals from the second breed form four groups. Fifteen RAPD primers were initially selected and applied in other study by Jawasreh *et al* (10), among them seven were chosen for further analysis to the genetic relatedness among four local breeds of goat, based on band pattern quality, reproducibility, and the presence of distinctive bands. Genetic distances between populations as computed from combined data for all seven primers ranged from 0.0165 to 0.0091. Phylogenetic relationship among these lines and breeds revealed two main clusters. Although the application of PCR-based technique is become so common in the investigation of genetic diversity in biosystems, our libraries still lack of studies undertaken to reveal the genetic discrimination of goat animals in the country. Therefore, this study represents an opportunity to evaluate the genetic variation on DNA level among local goat breeds with the aid of RAPD-PCR technique.

Materials and Methods

A total of twelve blood samples, three from each of the four goat breeds were collected. Samples were kept uncoagulated with Acid citrate dextrose (ACD) and stored frozen in the laboratory till it was subjected to DNA extraction. Total genomic DNAs were extracted from blood samples by using QIAgen Ampli Kit (QIAgen-Germany) following the procedure described by the manufacturer. DNA concentration was determined spectrophotometrically, and the quality of DNA was checked by 0.8% agarose gel electrophoresis. The following formulas were used to estimate DNA concentration and purity ratio, respectively:

- *DNA concentration (µg/ml) = O.D. 260nm x 50 x Dilution factor*
- *DNA purity ratio = O.D.260/O.D.280*

Then, DNA samples were diluted to the final concentration of 50ng. A total of 16 decamer oligonucleotide primers (Table 1), (Operon Technologies-USA) with 60-70% GC content were used in order to disclose the genetic relationship among goat breeds.

Table (1) RAPD primers sequences used to amplify local goat DNA

DNA primers code	Sequence *5'→3'*	DNA primers code	Sequence *5'→3'*
A3	AGTCAGCCAC	**C5**	GATGACCGCC
A10	GTGATCGCAG	**C12**	TGTCATCCCC
A11	CAATCGCCGT	**C20**	ACTTCGCCAC
A18	AGGTGACCGT	**D15**	CATCCGTGCT
B5	TGCGCCCTTC	**D20**	ACCCGCTCAC
B8	GTCCACACGG	**E4**	GTGACATGCC
B11	GTAGACCCGT	**E7**	AGATGCAGCC
C1	TTCGAGCCAG	**N16**	AAGCGACCTG

PCR reaction was performed with 200 ul sterilized microtubes. The final reaction mixture was 25µL (50ng genomic DNA, 1.25µl of 1X Taq buffer, 0.2mM dNTPs, 1.5 mM $MgCl_2$, 2µl random primer (10 pmol/µl) and 1.25U/µl Taq DNA polymerase. Deionized distilled water was used to complete the reaction volume to 25µL. By using Eppendorf Thermal Cycler (Mastercycler-Germany), tubes containing the reaction mixture were subjected to the thermal profile as detailed bellow: Initial denaturation step was performed at 94°C for 4 min followed by 45 cycles of: annealing at 36°C for 1 min; then extension step was accomplished at 72ºC for 2 min, followed by final extension at 72°C for 5 min, finally samples were stored at 4°C. The amplified DNA fragments were separated on 1.5% agarose gel and stained with ethidium bromide. Gel documentation system was used to visualize and document the amplified fragments. For analysis genetic diversity, discrete amplified fragments across the lanes were scored. Every scorable band was considered as

single allele/locus and was scored as present (1) or absent (0). Genetic similarity was calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedures as described by (11):

- Genetic Similarity (GS) = 2Nij/(Ni+Nj)

Where: *Gs=Genetic Similarity between two populations.*
Nij = Total number of loci (bands) in populations i and j.
Ni = Total number of loci (bands) in population i.
Nj = Total number of loci (bands) in population j.

Polymorphism was estimated according to the following formula:

- Polymorphism % = (Np/Nt)×100

Where: *Np=the number of polymorphic bands of primer.*
Nt= the total number of bands of same primer.

The bivariate data (1-0) and genetic similarity (GS) coefficient matrices of three goat populations based on the data of twelve RAPD primers were used to construct a dendrogram by using unweighted pair group method with arithmetic average (UPGMA) through MVSP (Multi Variate Statistical Package) computer software.

Results and Discussion

The results showed that the primers were varied in their ability to detect genetic variation among studied goat individuals as they showed different banding pattern across the DNA samples. Four primers were failed to amplify any fragment, in addition to two other primers succeeded in producing fragments but not in all of the individuals (results were not taken). Seven out of sixteen primers were amplified 69 fragments, 32 out of these (46.3%) were found to be polymorphic (Table 2). These results agreed with those obtained by Esmaeelkhanian *et al* (6), Xiang *et al* (7) and Yadav and Yadav (8). The number of amplified fragments was ranged from 8 in primers C5 and C12 to 13 in primer D15. Molecular size of amplified fragments was ranged from 0.16 kbp to 4.8 kbp.

Table (2) Scorable fragments of amplified goat DNA by seven RAPD primers

RAPD primers	Total number of fragments	No. of polymorphic fragments	Percent of polymorphism (%)	Size of fragments (kbp)
A3	9	5	56	0.3-2.4
A10	11	9	82	0.2-2.5
A18	9	3	33	0.16-4.8
C5	8	3	38	0.4-2.3
C12	8	4	50	0.3-2.3
D15	13	5	38	0.18-2.1
E7	11	3	27	0.2-3.9
Total	***69***	***32***	***46***	***0.16 - 4.8***

Nine fragments were generated by A3 primer (Fig.1), five (56%) of them were polymorphic. The amplified products ranged in molecular size between 0.3 kbp and 2.4 kbp. Primer A10 generated eleven bands with molecular size ranged from 0.2 to 2.5 kbp (Fig.1). The primer showed good ability in distinguishing between studied breeds as it produced 9 (82%) polymorphic fragments. The primer produced unique fragmenting patterns allowing the discrimination of the fourth and the ninth individuals against the others. These individuals lost fragment with the molecular size of 0.28 kbp. A18 primer amplified a total of nine fragments (Fig.2), with molecular size ranged between 0.16 kbp and 4.8 kbp, three of them were polymorphic, and hence computed percentage of polymorphism was (33%). A total of eight bands were amplified by C5 RAPD primer (Fig.2). The molecular size of these bands ranged from 0.4 to 2.3 kbp. The primer showed humble distinctness between individuals as it gained three (38%) polymorphic bands. The individual no. 7 and no.8 have lost one easily detected fragment at the molecular size of 0.6 kbp. The results cleared that primer C12 was owned one of the minimum values of the amplified fragments (Fig.3), which was 8 bands; half of them were polymorphic (50%).

Maximal molecular size of amplified fragment was 2.3 kbp, while the minimum was of 0.3 kbp. The primer lost its annealing loci in two individuals (7 and 8), which resulted in losing amplified fragment at the molecular size of 0.19 kbp. The highest amplified fragments which were 13 were scored by D15 primer (Fig.3), with molecular size ranged from 0.5 to 3.5 kbp. Five (38%) out of the amplified fragments were polymorphic. Primer E7 gave a total number of 11 fragments (Fig.4), three of them were polymorphic scoring polymorphism percentage of 27%. The molecular sizes of produced fragments ranged from 0.2 kbp to 3.9 kbp. The primer was able to characterize some individuals by amplifying unique bands with different molecular sizes; one of these was 3.9 kbp in size discriminate individuals no. 4 and 9.

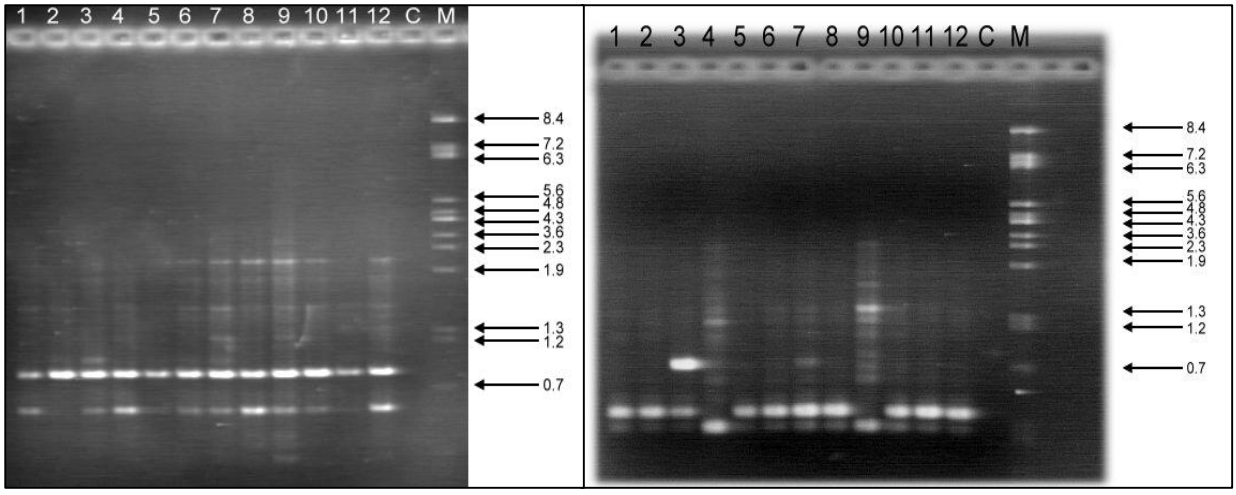

Fig. (1) PCR Amplified products of 12 goat individuals using RAPD primers A3 (left shape) and A10 (right shape). M= Molecular marker (Lambda DNA digested with *BstEII*): C= Control negative

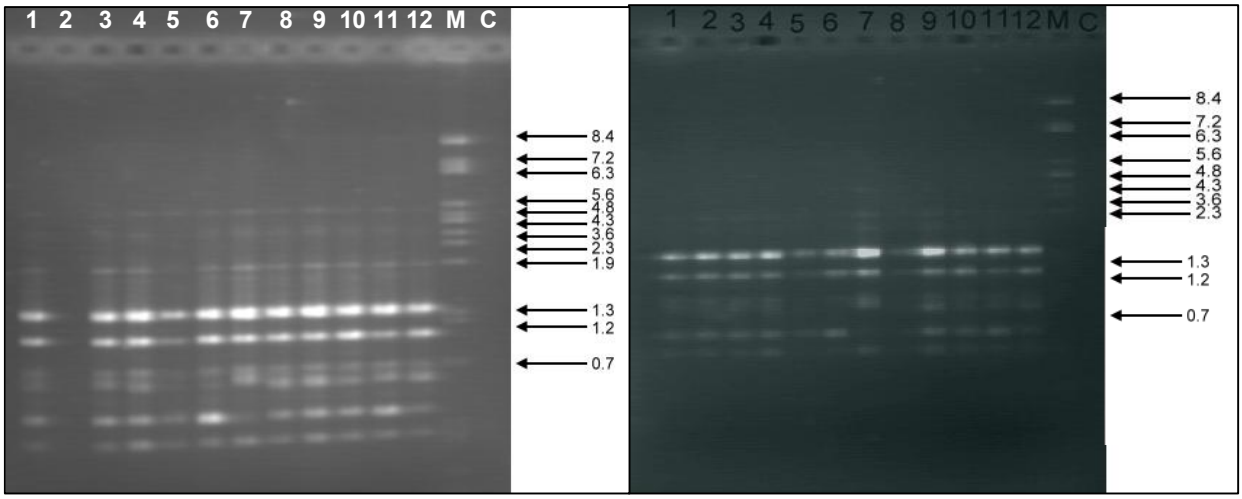

Fig. (2) PCR Amplified products of 12 goat individuals using RAPD primers A18 (left shape) and C5 (right shape). M= Molecular marker (Lambda DNA digested with *BstEII*): C= Control negative

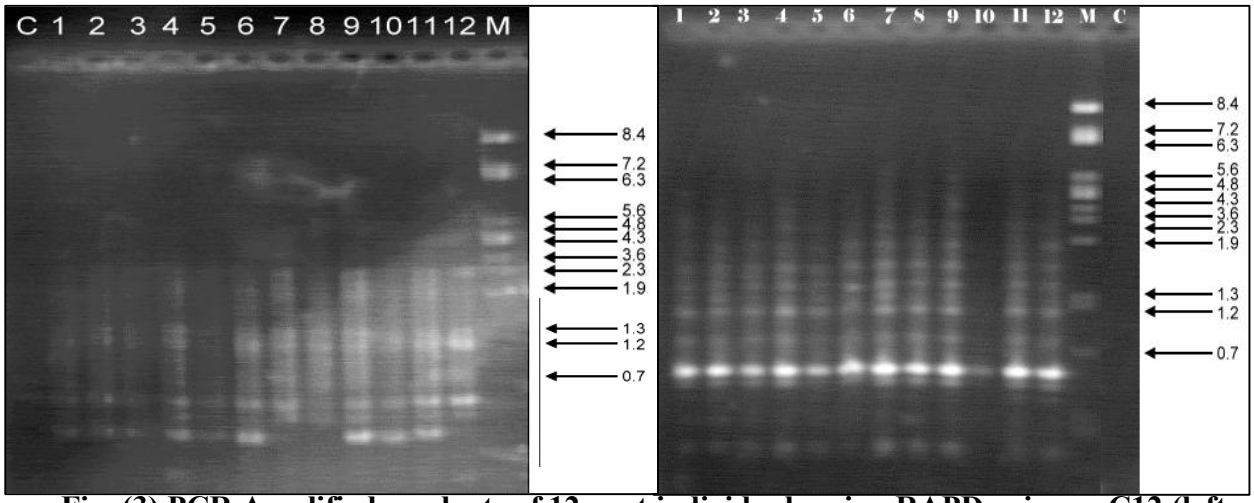

Fig. (3) PCR Amplified products of 12 goat individuals using RAPD primers C12 (left shape) and D15 (right shape). M= Molecular marker (Lambda DNA digested with *BstEII*): C= Control negative

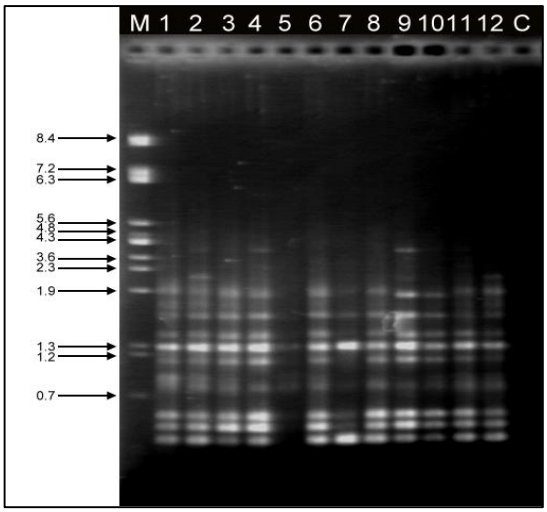

Fig. (4) PCR Amplified products of 12 goat individuals using RAPD primers E7. M= Molecular marker (Lambda DNA digested with *BstEII*): C= Control negative

The highest values of pair wise comparison of genetic similarity (Table 3) were between 4 and 9; 11 and 12 goat individuals (0.97% and 0.96%, respectively), while the lowest values of genetic similarity were between 5 and 9; 2 and 5 individuals (0.61% and 0.63%, respectively). Genetic similarity documented by Li *et al* (5) and Xiang *et al* (7) were within the same range. The individuals with high genetic similarity may be due to their descendness from the same ancestor and/or cross breeding within and between their ancestor populations. On the other side, low genetic similarity indicated that new genetic material still raise up even due to different rate of inbreeding within local goat populations, or it may be resulted from the different origin of these individuals.

Table (3) Average estimates of genetic similarity at DNA level among 12 individuals of local goat breed using 10 universal primers

Ind. No.	1	2	3	4	5	6	7	8	9	10	11	12
1	1.00											
2	0.84	1.00										
3	0.91	0.82	1.00									
4	0.85	0.78	0.86	1.00								
5	0.72	0.63	0.74	0.63	1.00							
6	0.90	0.78	0.90	0.87	0.71	1.00						
7	0.83	0.75	0.86	0.89	0.65	0.89	1.00					
8	0.86	0.69	0.87	0.80	0.69	0.90	0.83	1.00				
9	0.84	0.74	0.86	0.97	0.61	0.89	0.91	0.82	1.00			
10	0.92	0.82	0.92	0.88	0.68	0.91	0.90	0.86	0.90	1.00		
11	0.94	0.82	0.89	0.89	0.71	0.92	0.88	0.89	0.88	0.92	1.00	
12	0.92	0.78	0.89	0.89	0.67	0.93	0.92	0.92	0.90	0.93	0.96	1.00
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

Depending on banding pattern resulted from the using of RAPD primers, the dendrogram tree (Fig.5) was constructed. Goat individuals, as expected separated into four main clusters; I, II, III and IV. Cluster I was comprised from individual no.5; cluster II was comprised from individual no.2; cluster III was comprised from individuals no. 4, 7 and 9; cluster IV was comprised from individuals no. 3, 6, 10, 1, 12, 11 and 8.

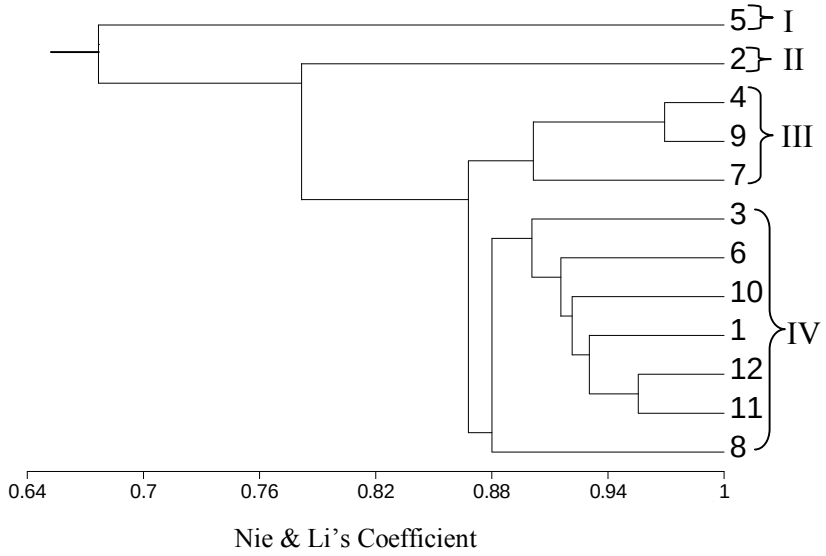

Fig. (5) Dendrogram obtained by cluster analysis of twelve individuals of local goat breeds based on fragment polymorphisms generated by seven RAPD primers

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