MOLECULAR GENETIC CHARACTERIZATION FOR TWO EGYPATIAN GOAT BREEDS

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Goat is widely distributed all over the world. They consider as one of the most important economic sources of meat and milk in developing countries. In Egypt, there are three major native goat breeds namely; Baladi, Barki (reared for meat production) and Zaraibi (reared for milk production). During the last three decades, classical strategies for evaluating genetic variability such as comparative anatomy, morphology, and embryology, have been increased. Nowadays, biochemical genetic fingerprints as well as molecular genetic fingerprint are quiet useful tools for genetic relationships studies among different resources (White and Coocke, 1992; Radovic and Vapa, 1996). Modern DNA manipulation provides unlimited potentialities for establishing accurate fingerprints at the molecular level. The development of "molecular markers" which are based on polymorphism found in protein or DNA has greatly facilitated researches in a variety of many biological branches such taxonomy and genetic relationships (Williams *et al*., 1990; Noli *et al*., 1997). It is highly recommended that biochemical and molecular genetic fingerprints would act as good tools for the characterization and identification of goat breeds. Molecular genetic markers and determination of genetic differences between breeds are helpful in the genetic breeding programs for the improvement of productive traits such as milk and meet (Amills *et al*., 1995). Based on the established fact that proteins are the other face of genetics, the biochemical assays of genetic variation at the protein molecular level can provide rich insights into the genetic structure of biological organisms (Elmasry and Asal, 2000).

MATERIALS AND METHODS

1. Materials

The base goat population used in the present study was assembled from Sakha and Bourg El-Arab Experimental Stations, belonging to Animal Production Research Institute, Agriculture Research Center (ARC). Ten does of different ages from Baladi and Barki breeds were chosen. Blood samples were collected from the jugular vein of each animal using vacutainer glass tubes which containing disodium ethylene diamine tetra acetic acid EDTA (EDTA-Na_2) as anticoagulant reagent. Blood plasma was obtained by centrifugation of blood samples at 10000

rpm for 10 minutes at 4°C, then plasma protein (supernatant) was transferred to clean plastic vials and stored at -20°C until electrophoretic analysis of protein. The pellet was immediately stored at -20°C for DNA extraction for PCR analysis.

2. Methods

2. 1. Biochemical Analyses

In SDS-PAGE, samples were applied to a 15% of polyacrylamide gel. Gel preparation, electrophoretic conditions, staining and destaining of gels were done according to Bollag and Edestein (1994). While in Native- PAGE electrophoresis, samples were applied to a 10% polyacrylamid gel according to Hames and Rickwood (1981).

2.2. RAPD Analysis

Genomic DNA was extracted from ten individuals in each breed according to Sambrook *et al*. (1989). The amplification conditions and PCR mixture were set according to Williams *et al*. (1990). A set of ten decamer random primers was used as listed in Table (1). The amplified products (12.5 ul loaded) were separated on 1.5% agarose gels.

2.3 Gel photographing

Protein gels were photographed after the distaining period, when the gel background became clear or relatively transparent. The DNA gels were immediately photographed using UV-based gel documentation system (Bio-Rad Gel Doc 2000 apparatus).

2.4 Data Analysis

All gels resulted from protein and DNA electrophoresis, were analyzed using TotalLab1.1 software to estimate the molecular weights and sizes of protein and DNA fragments, respectively. Then SPSS10 was used to calculate the similarity percentages between the studied goat breeds. The homogeneity percentages were estimated in the following manner: Homogeneity % = number of monomorphic bands/ total number of bands according to Lynch (1990).

RESULTS AND DISSCUSION

1. Genetic polymorphism of the tested goat breeds as detect by SDS-protein

SDS-protein of ten blood plasma samples of each breed was used to assess the genetic similarity within the studied breeds (Baladi and Barki).

The SDS-protein banding pattern for Baladi breed is shown in Fig. (1) and scored in Table (2). The total number of 14 bands was recorded which ranged from 204 to 8 KDa. There were only six polymorphic bands with molecular weights of 175, 165, 152, 61, 46 and 8 KDa, while the other bands were monomorphic. The bands frequencies were ranged from 0.3 to 1.0 with average of 0.88. The average of similarity value within this breed was 0.91 which indicated a high homogeneity value within this breed.

Figure (2) and Table (3) showed banding pattern for Barki breed. The total number of 15 bands was recorded which ranged from 202 to 9 KDa. There were only nine polymorphic bands with molecular weights of 180, 163, 146, 137, 123, 86, 66, 55 and 27 KDa, while the other bands were monomorphic. The bands frequencies were ranged from 0.4 to 1.0 with average of 0.77. The average of similarity value within this breed was 0.82 which indicated a moderate homogeneity value within this breed.

1.2. Genetic polymorphism of the studied goat breeds used by native-protein

Native-protein of ten blood plasma samples of each breed was used to characterize and differentiate within the studied goat breeds (Baladi and Barki). The native-protein banding pattern revealed the presence of up to six different band zones. These zones are immunoglobulin (γ-globulin), post-transferrin, transferrin (β-globulin), α-globulin (slow and fast), albumin and pre-albumin according to Mordacq and Roberta (1994).

The native-protein banding patterns of ten Baladi breed individuals are shown in Fig. (3) and scored in Table (4). The total number of bands was 13 bands with relative fronts ranged from 0.10 to 0.83. There was only one polymorphic band with relative front of 0.64 belonged to α-globulin zone, while the other bands were monomorphic. Therefore, the homogeneity percentage was about 92% in this breed.

Results which were illustrated in Fig. (4) and Table (5) showed the native-protein banding patterns of ten Barki breed individuals. The total number of bands was 14 bands with relative fronts ranged from 0.12 to 0.93. There was only one polymorphic band with relative front of 0.46 belonged to transferrin zone, while the other bands were monomorphic. However, the homogeneity percentage was about 93% in this breed.

The homogeneity percentage of native-protein banding patterns within Barki breed was 93%, while in the Baladi breed was 92%. The similarity matrix based on SDS-PAGE and Native-PAGE among the studied goat breeds was 0.91.

Many researchers employed classical biochemical polymorphic markers such as polymorphic proteins (Wang *et al*., 1990; Deza *et al*., 2000) to characterize and identify goat breeds, to study the genetic variation within goat breeds and to determine the genetic relationships among goat populations.

The assessment of genetic relationships among the studied goat breeds is quite important for the planning of future breeding programs. These results were in some agreements with those of Braend and Tucker (1982), which differentiated among some Indian breeds of goats by using the same techniques. Moreover, Tapio *et al*. (2002) also differentiated among nine sheep breeds of Finland and North-Western of Russia by the same techniques. Baker and Manwell (1991) estimated the genetic relationships among

European, Asian and African cattle breeds using the same techniques.

Concerning genetic relationships within studied breeds, recently many authors reflected this point such as Anous *et al*. (2008), who assessed the genetic structure within three Egyptian goat populations (Baladi, Barki and Zaraibi) using serum protein. They found that Barki population had the highest average value (0.69) followed by Baladi population (0.65), while Zaraibi population had the lowest value (0.55) and this may reflect a high degree of inbreeding in both Barki and Baladi populations compared to Zaraibi population. They concluded that protein analysis is a sensitive method for studying the genetic structure of goat population.

Concerning homogeneity percentages within studied breeds, Awad (2005) observed that the homogeneity percentages for Ossimi, Rahmani and Barki sheep breeds were 47, 41 and 29%, respectively. Ismail *et al*. (2006) measured variations in plasma protein among four camel breed (Fallahy, Magrnbi, Suclany and Mowaled) using protein native-gel electrophoresis. The results within and among this four breeds revealed the presence of six different fractions; immunoglobulin, post-transferrin, transferrin, α-globulin, albumin and post-albumin and found that homogeneity percentages were 10.0, 16.7, 31.6 and 20.8% for Maghraby, Sudany, Falahy and Mowaled, respectively.

3. RAPD analysis

Ten random primers (A7, C2, C5, C8, C16, C19, C20, UBC24, UBC92 and UBC93) were chosen to be used in the characterization and differentiation within the two studied goat breeds as shown in Figs (5 and 6).

RAPD analysis was used to estimate the genetic variations within the studied breeds using these ten primers to obtain a great number of PCR-amplified fragments. Similarity matrices resulted from using the ten primers was used to assess the genetic variations within these breeds as shown in Table (6). The similarity average values of each primer for the two tested breeds were varied from 0.69 (UBC 92 primer) to 0.95 (UBC 93 primer) which indicating the presence of various fragment polymorphisms for each tested primer. The similarity average values of each breed for all used primers were 0.85 and 0.83 for Baladi and Barki breeds, respectively, which indicates the presence of low genetic variations within the studied breeds. However, the similarity average value of the two tested breeds for all used primers was 0.84, which also indicating the presence of low genetic variations within the studied breeds.

Rahman *et al.* (2006) used eight random primers with 14 samples of black Bengal and Jamuna Pari goat breeds. The results of DNA analysis based on RAPD-PCR showed that genetic diversity mean was 0.3724 among the 14 goat breeds. The highest number of polymorphism was observed using primer BM1818. The

pairwise genetic distance values were ranged from 0.2500 to 1.000 in the dendrogram which indicated the segregation of the 14 goat breeds. Within Jamuna Pari goat, the genetic similarity was low as well as black Bengal goat. Recently, El-Badawy (2009) examined the DNA polymorphism in two goat breeds (Zaraibi and Damascus) using the RAPD-PCR technique. He found that this technique was able to separate with precision between Zaraibi and Damascus individuals using some specific bands of different molecular weights which produced by 10 selected primers. In general, A20, B08, C05 and C11 primers with the Zaraibi breed and A20 and C08 primers with the Damascus breed gave the highest numbers of polymorphic bands. These primers could be used to characterize such breeds.

SUMMARY

Two Egyptian goat breeds (Baladi and Barki) were characterized using biochemical (SDS-PAGE and Native-PAGE) and molecular genetics (RAPD-PCR) techniques. Ten individuals from each breed were blood sampled. SDS-protein profiles for Barki breed showed lower percentage of similarity (82%) than Baladi breed (91%) within each one of them, while Native-PAGE analysis showed a high homogeneity value within each of the studied goat breeds. Moreover, the mean of the band frequencies averages within Baladi and Barki goat breeds based on SDS-PAGE were 0.88 and 0.77, respectively. RAPD analysis using 10 random primers showed high similarity average values of each breed for all used primers which were 0.85 and 0.83 for Baladi and Barki breeds, respectively, which indicates the presence of low genetic variations within the studied breeds. However, the similarity average value of the two tested breeds for all used primers was 0.84, which also indicating the presence of low genetic variations within the studied breeds.

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Table (1): Codes, sequences and GC percentage contents of the used primers.

Primer code	Primer sequence	GC% content
A7	5'-GAAACGGGTG-3'	70
C2	5'-GTG AGG CGTC-3'	70
C5	5'-GATGACCGCC-3'	60
C8	5'-TGGACCGGTG-3'	70
C16	5'-CACACTCCAG-3'	60
C19	5'-GTTGCCAGCC-3'	70
C20	5'-ACTTCGCCAC-3'	60
UBC24	5'-ACAGGGGTGA-3'	60
UBC92	5'-CCTGGGCTTT-3'	60
UBC93	5'-GGGGGGAAAG-3'	70

Table (2): SDS-protein banding patterns scoring of Baladi breed individuals.

Band no.	MW (KDa)	BF	10	9	8	7	6	5	4	3	2	1
1	204	1.0	1	1	1	1	1	1	1	1	1	1
2	175	0.9	1	0	1	1	1	1	1	1	1	1
3	165	0.8	0	0	1	1	1	1	1	1	1	1
4	152	0.8	0	0	1	1	1	1	1	1	1	1
5	140	1.0	1	1	1	1	1	1	1	1	1	1
6	124	1.0	1	1	1	1	1	1	1	1	1	1
7	98	1.0	1	1	1	1	1	1	1	1	1	1
8	80	1.0	1	1	1	1	1	1	1	1	1	1
9	70	1.0	1	1	1	1	1	1	1	1	1	1
10	61	0.8	0	0	1	1	1	1	1	1	1	1
11	46	0.3	1	0	1	0	0	1	0	0	0	0
12	31	1.0	1	1	1	1	1	1	1	1	1	1
13	24	1.0	1	1	1	1	1	1	1	1	1	1
14	8	0.7	0	1	1	0	0	1	1	1	1	1

MW: molecular weight BF: band frequency 1= present band 0= absent ban

Table (3): SDS-protein banding pattern scoring of Barki breed individuals.

Band no.	MW (KDa)	BF	10	9	8	7	6	5	4	3	2	1
1	202	1.0	1	1	1	1	1	1	1	1	1	1
2	180	0.5	1	1	0	0	0	1	1	1	0	0
3	163	0.6	1	1	1	0	0	1	1	1	0	0
4	146	0.8	1	1	1	1	1	1	1	1	0	0
5	137	0..4	0	0	0	0	0	0	1	1	1	1
6	123	0.8	1	1	1	1	1	1	1	1	0	0
7	95	1.0	1	1	1	1	1	1	1	1	1	1
8	86	0.7	1	1	1	0	1	1	1	1	0	0
9	79	1.0	1	1	1	1	1	1	1	1	1	1
10	66	0.4	0	0	0	0	1	1	1	0	0	1
11	55	0.6	1	1	0	0	1	1	1	0	1	0
12	45	1.0	1	1	1	1	1	1	1	1	1	1
13	31	1.0	1	1	1	1	1	1	1	1	1	1
14	27	0.7	1	1	1	1	1	1	1	0	0	0
15	9	1.0	1	1	1	1	1	1	1	1	1	1

MW: molecular weight BF: band frequency 1= present band 0= absent ban

Table (4): Score of native-protein banding patterns of Baladi breed individuals.

Band no.	Fraction	RF	10	9	8	7	6	5	4	3	2	1
1	Immunoglobulin	0.10	1	1	1	1	1	1	1	1	1	1
2		0.16	1	1	1	1	1	1	1	1	1	1
3		0.20	1	1	1	1	1	1	1	1	1	1
4	Post-Transferrin	0.23	1	1	1	1	1	1	1	1	1	1
5	Transferrin	0.35	1	1	1	1	1	1	1	1	1	1
6		0.38	1	1	1	1	1	1	1	1	1	1
7		0.42	1	1	1	1	1	1	1	1	1	1
8		0.45	1	1	1	1	1	1	1	1	1	1
9		0.53	1	1	1	1	1	1	1	1	1	1
10	α-globulin	0.59	1	1	1	1	1	1	1	1	1	1
11		0.64	0	1	1	1	1	1	1	1	0	0
12	Albumin	0.71	1	1	1	1	1	1	1	1	1	1
13	pre- Albumin	0.83	1	1	1	1	1	1	1	1	1	1

1: present 0: absent RF: relative front

Table (5): Score of native-protein banding patterns of Barki breed individuals.

Band no.	fraction	Rf	10	9	8	7	6	5	4	3	2	1
1	Immunoglobulin	0.12	1	1	1	1	1	1	1	1	1	1
2		0.18	1	1	1	1	1	1	1	1	1	1
3		0.21	1	1	1	1	1	1	1	1	1	1
4	Post-Transferrin	0.27	1	1	1	1	1	1	1	1	1	1
5	Transferrin	0.32	1	1	1	1	1	1	1	1	1	1
6		0.42	1	1	1	1	1	1	1	1	1	1
7		0.46	1	1	1	1	1	1	1	1	0	1
8		0.48	1	1	1	1	1	1	1	1	1	1
9		0.50	1	1	1	1	1	1	1	1	1	1
10	α-globulin	0.61	1	1	1	1	1	1	1	1	1	1
11		0.67	1	1	1	1	1	1	1	1	1	1
12		0.74	1	1	1	1	1	1	1	1	1	1
13	Albumin	0.80	1	1	1	1	1	1	1	1	1	1
14	pre- Albumin	0.93	1	1	1	1	1	1	1	1	1	1

1: present 0: absent RF: relative front

Table (6): Average of similarity values within each of Baladi and Barki breeds of each used primer.

Breeds	Primers										
	A7	C2	C5	C8	C16	C19	C20	UBC 24	UBC 92	UBC 93	Breed Averages
Baladi	0.91	0.82	0.86	0.76	0.75	0.73	0.95	0.96	0.80	0.94	0.85
Barki	0.92	0.77	0.92	0.73	0.69	0.92	0.87	0.91	0.58	0.96	0.83
Primer Averages	0.915	0.795	0.89	0.745	0.72	0.825	0.91	0.935	0.69	0.95	0.84

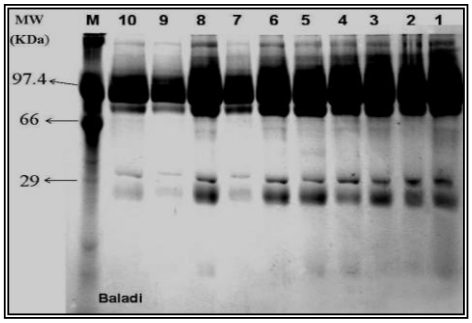

Fig. (1): SDS-protein banding pattern of prolific Baladi breed individuals (1-10).

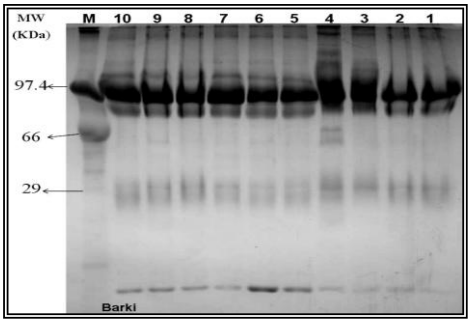

Fig. (2): SDS-protein banding pattern of non-prolific Barki breed individuals

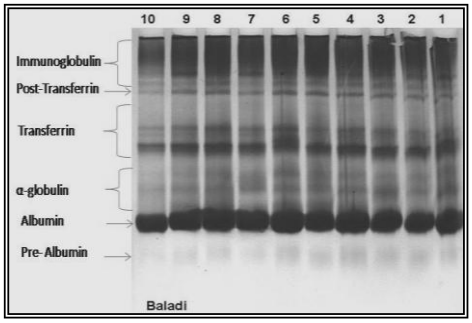

Fig. (3): Native-protein banding patterns of Baladi breed individuals.

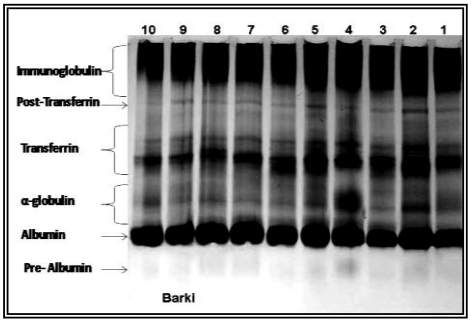

Fig. (4): Native-protein banding patterns of Barki breed individuals

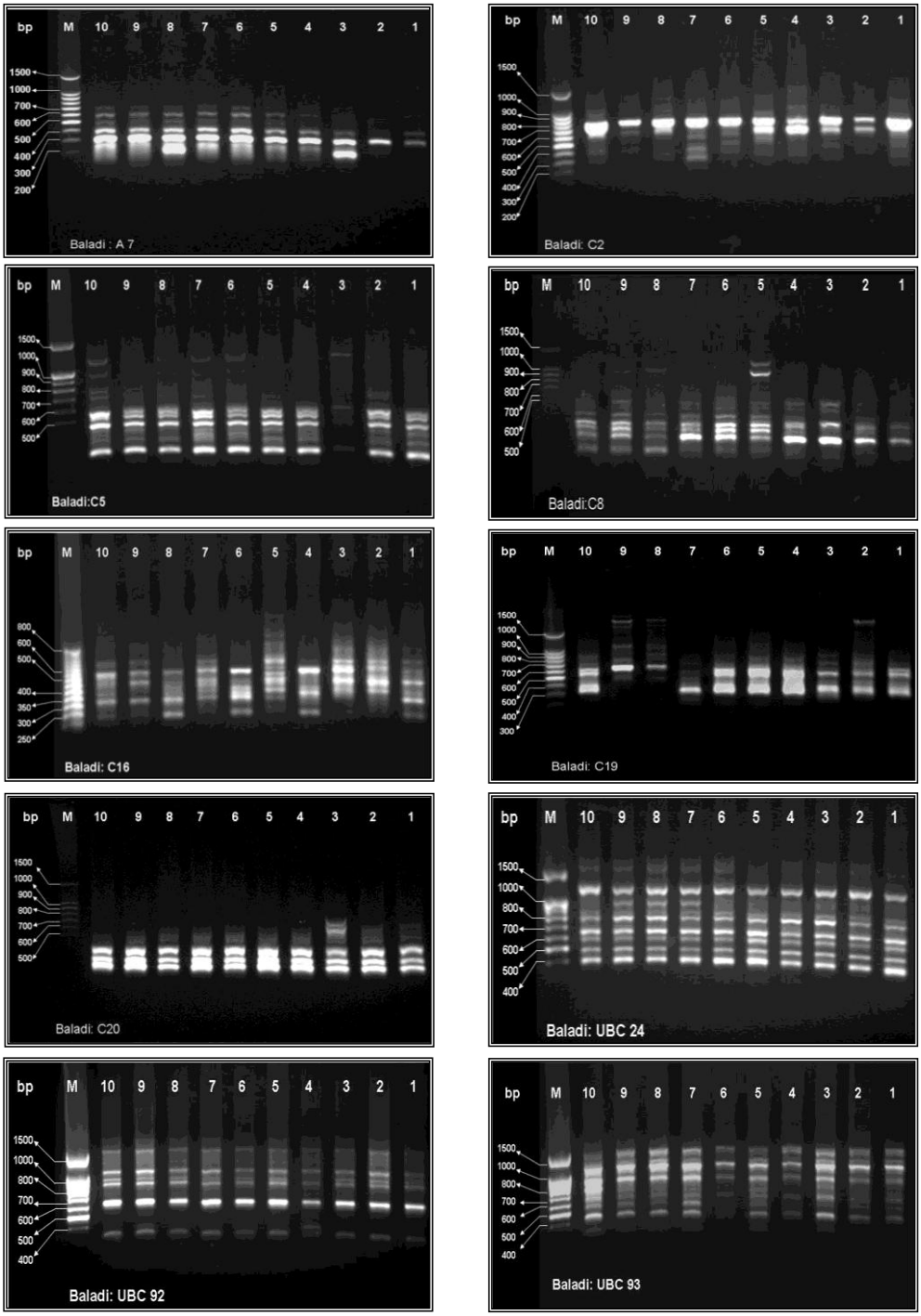

Fig. (5): RAPD-PCR fragments produced using ten individuals samples for Baladi breed against the ten tested primers.

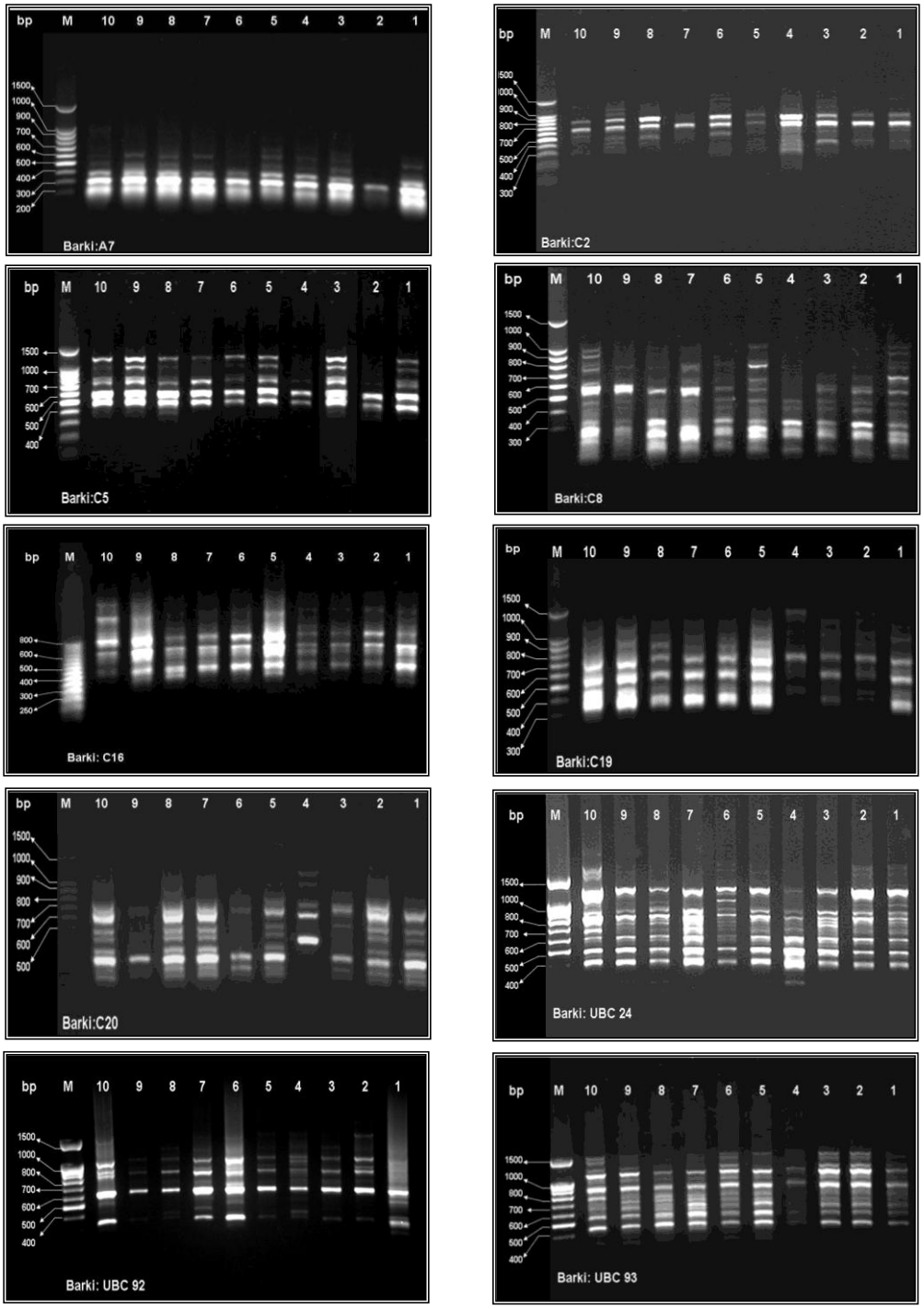

Fig. (6): RAPD-PCR fragments produced using ten individuals samples for Barki breed against the ten tested primers.