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ORIGINAL ARTICLES

Molecular Genetic Studies for Damascus Goat Breed Raised Under Egypatian Condtions

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Abstract: Syrian (*Damascus*) goat breed was characterized using biochemical (SDS-PAGE and Native-PAGE) and molecular genetic (RAPD-PCR) techniques. This breed was classified into prolific and non-prolific groups according to litter size trait. Ten does from each group were blood sampled. The similarity averages within each of prolific and non-prolific Damascus goat groups, based on SDS-protein profiles, were 0.94 and 0.98, respectively. This indicates a high homogeneity value within each one. While native-protein analysis showed a lower homogeneity value within each one of prolific and non-prolific Damascus goat groups. These were 71 and 66 %, respectively. RAPD analysis, using 10 random primers, showed high similarity average values of each group for all used primers. These were 0.83 and 0.84 for prolific and non-prolific Damascus goat groups, respectively. This indicates the presence of low genetic variations within this breed.

Key words: Genetic Characterization, Polymorphism, Similarity, homogeneity, Damascus Goat Breed.

Introduction

Goats are the most versatile domestic animals in adaptation to arid and humid, tropical and cold, and desert and mountain conditions (Gall, 1991; Quarterium, 1991 and Silanikove, 2000). The Damascus goat, also known as Shami, is a native breed of Syria and other Near East countries. The Damascus goat has a reddish brown coat colour consisting mostly of long hair. The ears are long and an adult live weight of about 65±5 kg for the female and 75±5 kg for the male. The head is long with a Roman nose and the presence of horns in both sexes is associated with inter-sexuality (Hancock and Louca, 1975). The breed is considered as one of the best dual-purpose breeds of the Middle East under semi-intensive or intensive production systems, combining high prolificacy with high milk production (Hadjipanayiotou, 1987). Fertility is medium to high (80% to 90%), a characteristic of most goat breeds with high milk production. The prolificacy of the breed is among the highest in the region averaging 1.80 kids per doe kidding (Constantinou, 1981; Constantinou *et al*., 1981). The Ministry of Agriculture in Egypt imported some bucks and does of the Damascus goat for crossing with the Barki goat to improve its meat production. Recently the crossing between Damascus and Baladi goat started for the same reason. This improved breed, that lives and performs in Egypt, requires an improved management and feeding environment to express its full genetic potential.

During the last three decades, classical strategies for evaluating genetic variability such as comparative anatomy, morphology, and embryology, were on the rise. Nowadays, biochemical genetic fingerprint 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). 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 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).

The purpose of this study is to characterize and identify the genetic variation within goat breeds, thus we can determine the genetic relationships among goat populations.

Materials and Methods

1. Materials:

The base goat population used in the present study was obtained in 2007 from Sakha Experimental Station, that belongs to the Animal Production Research Institute, Agriculture Research Center (ARC). Twenty does of

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different ages from Syrian Damascus breed were chosen and grouped into two groups (ten in each one) according to their prolificacy trait. This was based on litter size (i.e. number of kids born per parturition per female) and represented the prolific (DH: two kids or more) and non-prolific (DL: only one kid) does using the pedigree records. Blood samples were collected from the jugular vein of each animal (the 20 sampled animals) using vacutainer glass tubes which containing disodium ethylene diamine tetra acetic acid (Na_2-EDTA) as an anticoagulant reagent. Blood plasma was obtained by centrifugation of these blood samples at 10000 rpm for 10 minutes at 4°C. Plasma proteins (supernatant) was transferred to clean plastic vials and stored at -20°C until the electrophoretic analysis of protein was performed. The pellet was immediately stored at -20°C for DNA extraction for PCR analysis.

2. Methods:

2. 1. Biochemical Analyses:

SDS-PAGE electrophoresis of blood 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 all individuals in each group 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.

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

2.3 Data Analysis:

All gels resulted from protein and DNA electrophoresis, were analyzed using Total Lab1.1 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. SDS-PAGE of the Tested Goat Breeds:

SDS-protein of ten blood plasma samples of each group was used to assess the genetic similarity within the studied Damascus goat breed.

The SDS-protein banding pattern of the 10 prolific Damascus goats is shown in Figure (1) and scored in Table (2). A total number of 15 bands were obtained. Their molecular weight ranged from 224 to 12 KDa. There were only five polymorphic bands with molecular weights of 224, 187, 68, 57 and 12 KDa, while the other bands were monomorphic. The band frequencies ranged from 0.3 to 1.0 with an average of 0**.**87**.** The average similarity value within this breed was 0.94 which indicated a high homogeneity value within this group.

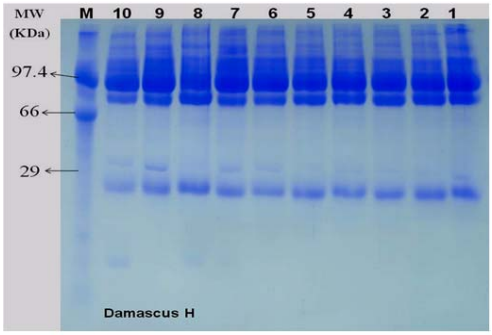

Fig. 1: SDS-protein banding pattern of the ten prolific Damascus goats.

Table 2: SDS-protein banding pattern of the ten prolific Damascus goats.

Band no.	MW (KDa)	BF	10	9	8	7	6	5	4	3	2	1
1	224	0.8	0	0	1	1	1	1	1	1	1	1
2	212	1.0	1	1	1	1	1	1	1	1	1	1
3	199	1.0	1	1	1	1	1	1	1	1	1	1
4	187	0.5	1	1	0	1	0	1	0	0	0	1
5	173	1.0	1	1	1	1	1	1	1	1	1	1
6	159	1.0	1	1	1	1	1	1	1	1	1	1
7	145	1.0	1	1	1	1	1	1	1	1	1	1
8	131	1.0	1	1	1	1	1	1	1	1	1	1
9	110	1.0	1	1	1	1	1	1	1	1	1	1
10	90	1.0	1	1	1	1	1	1	1	1	1	1
11	68	0.9	1	1	1	1	1	0	1	1	1	1
12	57	0.5	1	1	0	0	0	0	1	1	0	1
13	40	1.0	1	1	1	1	1	1	1	1	1	1
14	31	1.0	1	1	1	1	1	1	1	1	1	1
15	12	0.3	1	0	1	1	0	0	0	0	0	0

MW: Molecular weight. BF: Band frequency. 1= present band 0= absent band

The SDS-protein banding pattern of the 10 non-prolific Damascus goats is shown in Figure (2) and scored in Table (3). A total number of 17 bands were obtained. Their molecular weight ranged from 194 to 7 KDa. There were only two polymorphic bands with molecular weights of 183 and 48 KDa, while the other bands were monomorphic. The band frequencies ranged from 0.6 to 1.0 with an average of 0**.**97**.** The average similarity value within this breed was 0.98 which indicated an even higher homogeneity value within this group.

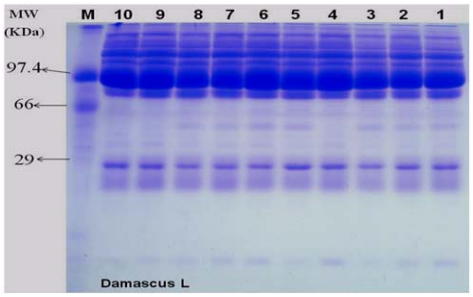

Fig. 2: SDS-protein banding pattern of the ten non-prolific Damascus goats.

2. Native-PAGE of the Studied Goat Breeds:

Native-protein of ten blood plasma samples from each group was used to characterize and differentiate the studied goat breed. 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 (Mordacq and Roberta, 1994).

The native-protein banding patterns for the prolific Damascus goats are shown in Figure (3) and scored in Table (4). The total number of bands was 14 bands with relative fronts ranging from 0.07 to 0.75. There were only four polymorphic bands with relative fronts of 0.36 and 0.45, belonged to the transferrin zone, and 0.48 and 0.60, belonged to the α-globulin zone, while the other bands were monomorphic. The homogeneity percentage was about 71% in this group.

Table 3: SDS-protein banding pattern of the non- prolific Damascus goats.

Band no.	MW (KDa)	BF	10	9	8	7	6	5	4	3	2	1
1	194	1.0	1	1	1	1	1	1	1	1	1	1
2	183	0.6	0	1	0	1	0	0	1	1	1	1
3	178	1.0	1	1	1	1	1	1	1	1	1	1
4	161	1.0	1	1	1	1	1	1	1	1	1	1
5	150	1.0	1	1	1	1	1	1	1	1	1	1
6	135	1.0	1	1	1	1	1	1	1	1	1	1
7	119	1.0	1	1	1	1	1	1	1	1	1	1
8	93	1.0	1	1	1	1	1	1	1	1	1	1
9	76	1.0	1	1	1	1	1	1	1	1	1	1
10	65	1.0	1	1	1	1	1	1	1	1	1	1
11	61	1.0	1	1	1	1	1	1	1	1	1	1
12	48	0.9	0	1	1	1	1	1	1	1	1	1
13	42	1.0	1	1	1	1	1	1	1	1	1	1
14	37	1.0	1	1	1	1	1	1	1	1	1	1
15	27	1.0	1	1	1	1	1	1	1	1	1	1
16	21	1.0	1	1	1	1	1	1	1	1	1	1
17	7	1.0	1	1	1	1	1	1	1	1	1	1

MW: Molecular weight. BF: Band frequency 1= present band 0= absent band

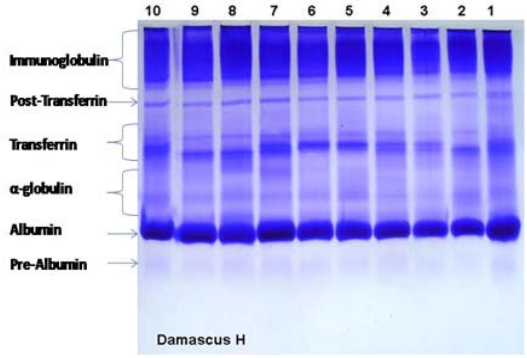

Fig. 3: Native gel-protein banding patterns of the ten prolific Damascus goats.

Table 4: Native-PAGE protein banding patterns of the ten prolific Damascus goats.

Band no.	fraction	Rf	10	9	8	7	6	5	4	3	2	1
1	Immunoglobulin	0.07	1	1	1	1	1	1	1	1	1	1
2		0.16	1	1	1	1	1	1	1	1	1	1
3		0.18	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	0.34	1	1	1	1	1	1	1	1	1	1
6		0.36	0	1	1	0	0	0	0	0	1	1
7		0.38	1	1	1	1	1	1	1	1	1	1
8		0.40	1	1	1	1	1	1	1	1	1	1
9		0.45	1	1	1	1	0	1	1	1	1	1
10	α-globulin	0.48	1	0	0	0	0	0	1	0	1	0
11		0.53	1	1	1	1	1	1	1	1	1	1
12		0.60	0	1	1	1	1	1	1	1	0	1
13	Albumin	0.64	1	1	1	1	1	1	1	1	1	1
14	pre- Albumin	0.75	1	1	1	1	1	1	1	1	1	1

1: present 0: absent RF: relative front

Results illustrated in Figure (4) and Table (5) showed the native gel-protein banding patterns for the ten non-prolific Damascus goats. The total number of bands was 15 bands with relative fronts ranging from 0.11 to 0.90. There were only six polymorphic bands with different relative fronts of 0.25 belonged to the immunoglobulin zone, 0.30 belonged to the post-transferrin zone, 0.47 and 0.56 belonged to the transferrin zone and 0.60 and 0.74 belonged to the α-globulin zone, while the other bands were monomorphic. The homogeneity percentage was about 60% in this group.

The homogeneity percentage of the native-protein banding patterns within the prolific Damascus breed group was 71% while it was 60% in the non-prolific Damascus breed group.

Many researchers employed classical biochemical polymorphic markers such as polymorphic proteins (Wang *et al*., 1990; Diez-Tàscon *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.

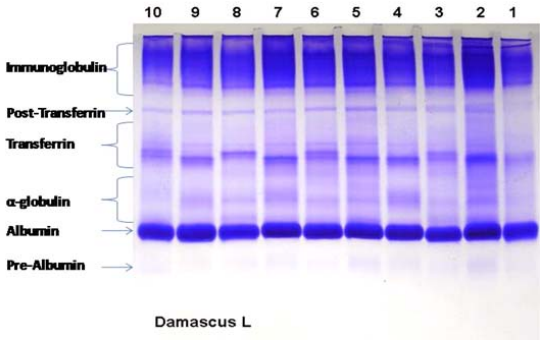

Fig. 4: Native gel-protein banding patterns of the non-prolific Damascus goats.

Table 5: Native-PAGE protein banding patterns of the non-prolific Damascus goats.

Band no.	Fraction	Rf	10	9	8	7	6	5	4	3	2	1
1	Immunoglobulin	0.11	1	1	1	1	1	1	1	1	1	1
2		0.22	1	1	1	1	1	1	1	1	1	1
3		0.25	1	1	1	1	1	1	1	1	1	0
4	Post-Transferrin	0.30	0	0	0	0	0	0	0	0	1	1
5	Transferrin	0.32	1	1	1	1	1	1	1	1	1	1
6		0.45	1	1	1	1	1	1	1	1	1	1
7		0.47	0	1	0	1	1	1	0	0	1	0
8		0.50	1	1	1	1	1	1	1	1	1	1
9		0.52	1	1	1	1	1	1	1	1	1	1
10	α-globulin	0.56	0	0	1	1	1	1	0	1	0	0
11		0.60	0	0	0	1	1	1	0	0	1	0
12		0.67	1	1	1	1	1	1	1	1	1	1
13		0.74	0	0	1	1	1	1	0	1	1	0
14	Albumin	0.79	1	1	1	1	1	1	1	1	1	1
15	post- Albumin	0.90	1	1	1	1	1	1	1	1	1	1

1: present band 0: absent band RF: relative front

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) who differentiated among some Indian breeds of goats by using the same techniques. Moreover, Tapio *et* a*l*. (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 groups as shown in Figures 5 and 6.

RAPD analysis was used to estimate the genetic variations within the studied groups using these ten primers to obtain a great number of PCR-amplified fragments.

Similarity matrices resulted from using the ten primers were used to assess the genetic variations within these groups as shown in Table (6). The similarity average values of each primer for the two tested groups varied from 0.65 (UBC 92 primer) to 1.00 (C2 primer). This indicates the presence of varied fragmens polymorphism for each tested primer. The similarity average values of each group for all used primers were 0.83

and 0.84 for prolific and non-prolific Damascus goat breed groups, respectively. This indicates the presence of low genetic variations within the studied breed.

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

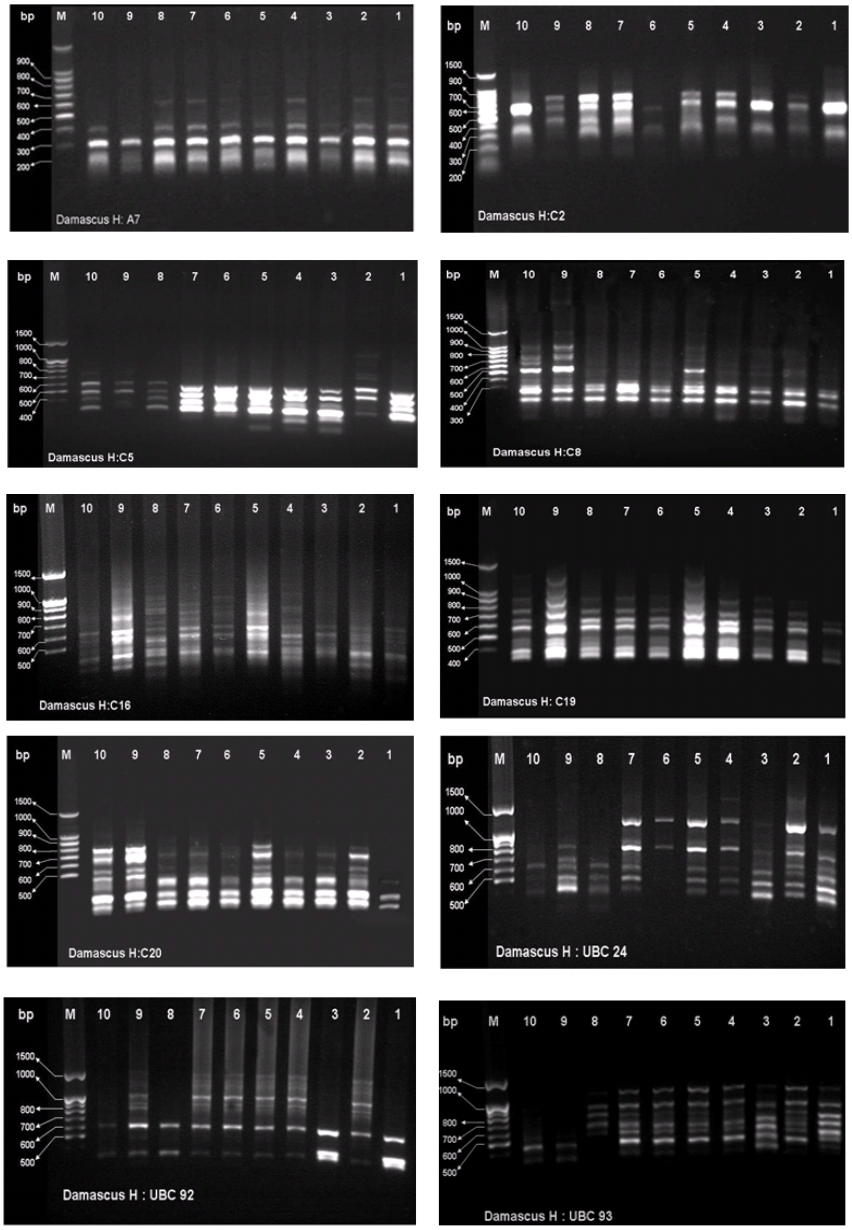

Fig. 5: RAPD-PCR fragments produced using ten individual samples from the prolific Damascus goat breed group against the ten tested primers.

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Table 6: Average similarity values within each of the prolific and the non-prolific Damascus goat breed groups for each of the ten used primers.

primers / Breeds	A7	C2	C5	C8	C16	C19	C20	UBC24	UBC92	UBC93	Breed Averages.
Prolific Damascus goats	0.96	1.00	0.83	0.70	0.74	0.90	0.90	0.71	0.65	0.87	0.83
Non-Prolific Damascus goats	0.85	0.82	0.80	0.87	0.76	0.96	0.96	0.88	0.68	0.90	0.84
Primer Averages.	0.905	0.91	0.815	0.785	0.75	0.93	0.93	0.795	0.665	0.885	

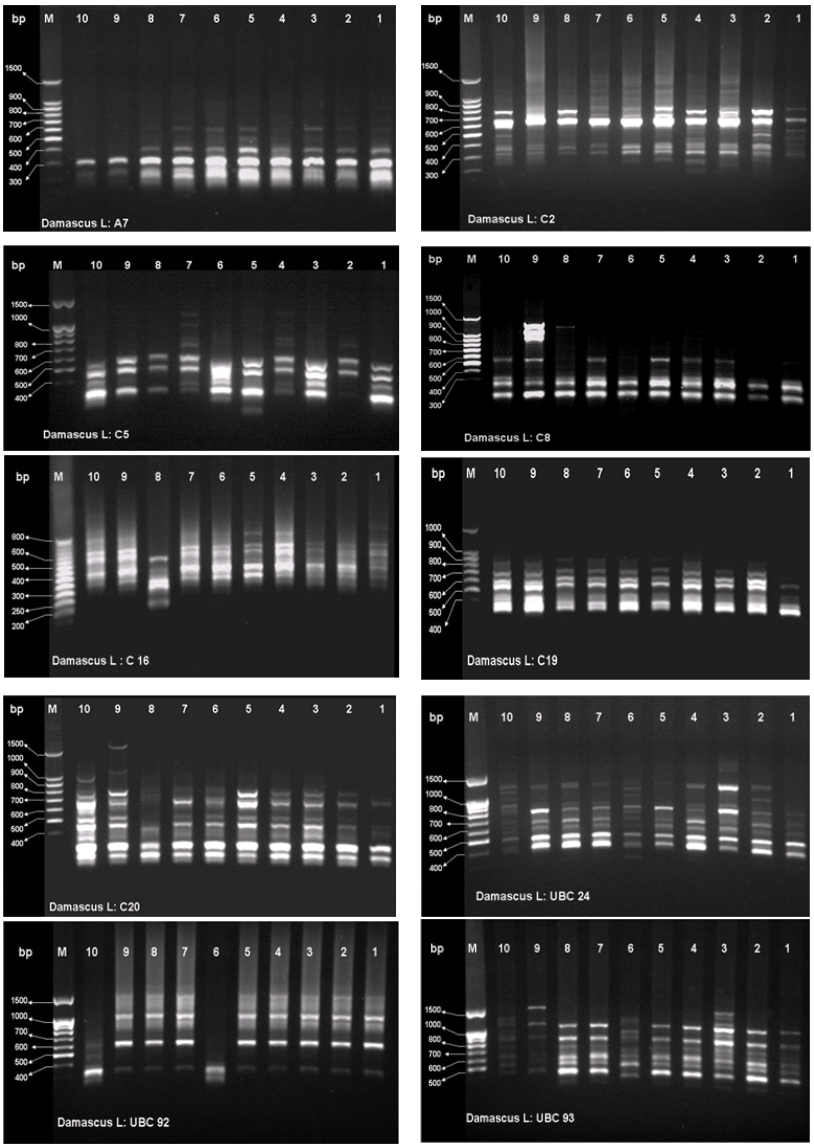

Fig. 6: RAPD-PCR fragments produced using ten individual samples from the non- prolific Damascus goat breed group against the ten tested primers.

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