ISOLATION AND CHARACTERIZATION OF ACROSIN GENE (*Acr*), EXON 5 IN THE BUCKS OF GOAT BREEDS

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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). Highest resolution of DNA variation can be obtained using sequence analysis that provides the fundamental structure of gene systems. DNA sequencing is generally not practical to identify variation between animals for the whole genome, but is a vital tool in the analysis of gene structure and expression (Drinkwater and Hazel, 1991). Advances in the understanding of the physiology, cell biology and bio-chemistry of reproduction have facilitated genetic analyses of fertility. Currently there are more than 200 known genes that are involved in the production of fertile sperm cells. The completion of a number of mammalian genome projects will aid in the investigation of these genes in different species. Great progress has been made in the understanding of genetic aberrations that lead to male infertility. Additionally, the first genetic mechanisms are being discovered that contribute to the quantitative variation of fertility traits in fertile male animals (Leeb *et al*., 2005). The reproductive gene *Acr* encodes acrosin, a multifunctional protein that is abundant in the acrosomal compartment of the sperm head, which released during the early stages of the acrosome reaction, mediates secondary or consolidated binding of spermatozoa to the zona pellucida by virtue of its carbohydrate-binding capacity (Jones *et al*., 1988). Acrosin is considered to play an essential role in fertilization, recognition, binding and penetration of the zona pellucida of the ovum (Klemm *et al*., 1991). Acrosin is thought to be important for secondary binding of sperm to the egg zona pellucida and subsequent proteolysis of the extracellular egg coat. Because a mouse knockout of Acr remained fertile, the functional significance of acrosin has been questioned (Baba *et al*., 1994). However, penetration of the zona pellucida in acrosin-deficient mice was significantly delayed, and there is ample evidence that acrosin is released during the acrosome reaction, binds the zona pellucida, and is critical for dispersion of acrosomal contents (Furlong *et al*., 2005; Tranter *et al*., 2000; Urch and Patel, 1991). A previous comparative analysis of Acr sequences from 10 mammal species revealed some evidence for adaptive change in this molecule, but P-values were

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marginal in comparisons of neutral models to selection models (Swanson *et al.*, 2003). Furthermore, large divergences between many of the sequences prevented comparison of the most rapidly evolving segment of Acr, exon 5 (Adham *et al.*, 1996; Zahn *et al.*, 2002). The bovine and porcine genes were cloned and characterized. Alignment of the intron/ exon structure of both genes with the previously characterized human, rat and mouse genes and with other serine protease genes reveals that the coded sequence of the mammalian proacrosin is distributed in 5 exons and the splice junction types are identical to the exons encoding the catalytic domain of other serine protease genes. A comparison of the bovine, porcine, human, guinea pig, rabbit, and rat and mouse preproprotein sequences shows that the catalytic domain is highly conserved, while the sequence of the proline rich domain is very variable among the species, ranging from 28.9% to 68.8% (Adham *et al.*, 1996).

MATERIALS AND METHODS

1. Materials

The base goat population used in the present study was assembled at the year of 2010 from Sakha and Bourg El-Arab Experimental Stations, belonging to Animal Production Research Institute, Agriculture Research Center (ARC). It consisted of 5, 5, 10 and 10 bucks of different ages from Baladi, Barki, Zaraibi and Syrian Damascus breeds, respectively. 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. Centrifugation of these blood samples at 10000 rpm for 10 minutes at 4°C, were carried out then plasma protein (supernatant) was transferred to clean plastic vials and stored at -20°C for other studies. The pellet was immediately stored at -20°C for DNA extraction and PCR analysis.

2. Methods

2.1. DNA Extraction from frozen blood

Genomic DNA was extracted from all individuals in each breed by modification of the method described by Sambrook *et al.* (1989).

2.2. Detection of Acrosin gene, exon 5 by Polymerase Chain Reaction

To identify the acrosin gene *Acr*, exon 5, two specific primers were designed based on the known sequence of exon5 of acrosin gene (*Acr*) in *Oreamnos americanus* goat breed found at NCBI database. Forward primer was 5'-GATAGGTTCTACCGCGGTGC-3' and reverse primer was 5'-GGAGGCATGTCGTCCTGGAA-3'.

PCR reaction was prepared in 10 µl contained: 2 µl DNA, 1 µl forward primer, 1 µl reverse primer, 1 µl dNTPs, 1 µl $MgCl_2$, 2 µl 5 X Green Flexi buffer, 1.9 µl H_2O (D.W) and 0.1 µl Taq polymerase. PCR reaction was achieved in the Techne thermocyclers which programmed as follows: Concerning the first cycle (initial denaturing), the denaturation temperature degree was 94°C for 4 min, and for the next 35 cycles, the denaturation, annealing and extension temperature degrees were 94°C for 45 sec, 58°C for 1 min and 72°C for 2 min, respectively, and for the last cycle (final extension), the temperature degree was 72°C for 5 min. Then, stored at 8°C for 10 min. *GelAnalyzer ver. 3* program was used to analyze the gels photos by comparing bands size with standard bands (DNA ladder).

2.3. DNA sequencing and analysis

One DNA sample from each breed individuals was randomly chosen and sent to Spain to be sequenced, then cleaned and concentrated using Fermentas (GeneJET PCR Purification Kit #K0702). These fragments were sequenced by sequencing service (Secugen S. L. of Madrid, Spain). The four DNA fragments sequences for *Acr* gene, exon5 which represented the four studied goat breeds were aligned using BioEdit program through multiple sequence alignment.

RESULTS AND DISSCUSION

1. Detection of acrosin gene (Acr) in the goat breeds

Profiles resulting from the use of two specific primers of *Acr* gene with the applied breeds are presented in Fig. (1). One band with a fragment size of a 378 bp was obtained in all individual samples for all breeds; this band represents exon 5 in *Acr* gene.

2. Acr gene, exon 5 DNA sequencing

One DNA sample from each goat breed with molecular size of 378 bp was randomly chosen and then sequenced through the automated DNA sequencing reaction with specific primers by sequencing service (Secugen S. L. of Madrid, Spain) as shown in Figs (2, 3, 4 and 5).

The number of each nucleotide in each sequenced fragment among these breeds was summarized in Table (1), where the numbers of Adenine and guanine bases were constantly in all breeds and were 78 and 73 bp respectively. While the number of cytosine base was 154 bp for Baladi and Zaraibi breeds and 155 bp for Barki and Damascus breeds. Also, the number of thymine base was 73 bp for Baladi and Zaraibi breeds and 72 bp for Barki and Damascus breed. Moreover, the number of G+C was 227 bp for Baladi and Zaraibi breeds and 228 bp for Barki and Damascus breeds. While the number of A+T was 151 for Baladi and Zaraibi breeds and 150 bp for Barki and Damascus breeds.

3. Acr gene, exon 5 DNA sequence alignment

The obtained sequences for *Acr* gene, exon 5 in the four studied goat breeds were compared with each other using BioEdit program as shown in Fig. (6). The sequences in Baladi and Zaraibi breeds were identity and differ from the sequences in Damascus and Barki breeds (who also identity) by only one base pair in a position number of 266 (T in Baladi and Zaraibi breeds while it was C in Barki and Damascus breeds).

The sequences of *Acr* gene of the four studied breeds were also compared by alignment with the sequences of *Acr* gene, exon 5 of other origins (Table 2) on the data base at the national center for biotechnology information (NCBI) website http://www.ncbi.nlm.nih.gov/. using BioEdit program as shown in Fig. (7).

Different alignment similarity values were obtained in Table (3) which showed that the sequences of *Acr* gene, exon 5 in the four studied goat breeds were similar to the sequences of *Acr* gene, exon 5 in the other origins especially with two kinds of sheep (*Ovis canadensis* and *Ovis dalli*) and with the *Oreamnos americanus* goat. Similarity values were ranged from 0.78 to 1.

The constructed dendrogram which illustrated the genetic relationships among the four studied breeds and other 20 origins is shown in Fig. (8). The 24 different *Acr* gene, exon5 sequences including the obtained sequences in this study were divided into many groups and each group was further divided into some subgroups. The results confirmed that the sequences of *Acr* gene, exon5 in the four studied goat breeds, two kinds of sheep (*Ovis canadensis* and *Ovis dalli*) and *Oreamnos americanus* goat were included in one group.

It is concluded from this study that the *Acr* gene, exon5 found in all studied goat breeds had a fragment with molecular size of a 378 bp and this fragment had conservation sequence in these breeds and sequence in Baladi and Zaraibi breeds was identical and differ from sequence of Damascus and Barki breeds (who also identical) by one base pair number 266 (T in Baladi and Zaraibi breeds while it was C in Barki and Damascus breeds). In addition, multiple alignment of Acr gene, exon 5 DNA sequence with the same DNA sequence in other origins give high similarity values. These results were in contrast with those of Gatesy and Swanson (2007) who reported that the *Acr* gene could be informative phylogenetic markers. In bovid artiodactyls (cattle, sheep, goats, andantelopes), exon 5 of *Acr* was highly variable, was characterized by a low level of homoplasy, and had a fairly even spread of substitutions across the 3 codon positions.

SUMMARY

Three Egyptian (Baladi, Barki and Zaraibi) and one Syrian (Damascus) goat breeds were used to isolate and characterize acrosin gene, exon 5. A sample of ten bucks individuals from Zaraibi and Damascus breeds and five bucks individuals from Baladi and Barki breeds were randomly taken and DNA was extracted from each individual and analyzed with the acrosin specific primers. One band with a fragment size of a 378 bp in all individual samples for all breeds represent exon 5 of *Acr* gene was obtained. Sequence analysis showed that Baladi and Zaraibi breeds were identical and differ from sequences

of Damascus and Barki breeds (who also identical) by one base pair number 266 (T in Baladi and Zaraibi breeds while it was C in Barki and Damascus breeds). The genetic analysis for *Acr* gene, exon 5 showed that this region was conserved among the studied goat breeds.

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Table (1): The numbers of A, C, G and T nucleotide for each chosen fragment of the four goat breeds.

	Number of nucleotides					
	A	C	G	T	G+C	A+T
Baladi	78	154	73	73	227	151
Barki	78	155	73	72	228	150
Zaraibi	78	154	73	73	227	151
Damascus	78	155	73	72	228	150

Table (2): *Acr* gene, exon 5 with its accession number and size in different origins at Gene Bank site.

	Source	The accession number	Name	Size (bp)
1	B. taurus	X68212.1	*Acr* gene, exon 5	539
2	Ovis canadensis Sheep	EF033138.1	*Acr* gene, exon 5	393
3	Ovis dalli Sheep	EF033137.1	*Acr* gene, exon 5	393
4	Gazella granti	EF033151.1	*Acr* gene, exon 5	408
5	Neotragus moschatus	EF033155.1	*Acr* gene, exon 5	408
6	Raphicerus campestris	EF033152.1	*Acr* gene, exon 5	408
7	Antidorcas marsupialis	EF033150.1	*Acr* gene, exon 5	408
8	Saiga tatarica	EF033152.1	*Acr* gene, exon 5	408
9	Tragulus napu	EF033156.1	*Acr* gene, exon 5	408
10	Pelea capreolus	EF033149.1	*Acr* gene, exon 5	408
11	Connochaetes taurinus	EF033147.1	*Acr* gene, exon 5	408
12	Beatragus hunteri	EF033146.1	*Acr* gene, exon 5	408
13	Alcelaphus buselaphus jacksoni	EF033145.1	*Acr* gene, exon 5	408
14	Oreotragus oreotragus	EF033154.1	*Acr* gene, exon 5	406
15	Damaliscus lunatus lunatus	EF033143.1	*Acr* gene, exon 5	406
16	Hippotragus niger	EF033142.1	*Acr* gene, exon 5	393
17	Oryx dammah	EF033141.1	*Acr* gene, exon 5	408
18	Damaliscus pygargus phillipsi	EF033144.1	*Acr* gene, exon 5	408
19	Connochaetes gnou	EF033148.1	*Acr* gene, exon 5	393
20	Oreamnos americanus Goat	EF033140.1	*Acr* gene, exon 5	393
21	Damascus Goat	-	*Acr* gene, exon 5	378
22	Barki Goat	-	*Acr* gene, exon 5	378
23	Zaraibi Goat	-	*Acr* gene, exon 5	378
24	Baladi Goat	-	*Acr* gene, exon 5	378

Table (3): Similarity matrix among the four studied breeds and other 20 origins based on *Acr* gene, exon 5 alignment.

Origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	ID	0.91	0.91	0.92	0.93	0.90	0.91	0.91	0.81	0.91	0.93	0.93	0.92	0.92	0.93	0.93	0.94	0.92	0.93	0.92	0.91	0.91	0.90	0.90
2		ID	1.00	0.90	0.90	0.88	0.89	0.89	0.79	0.89	0.95	0.94	0.94	0.90	0.94	0.93	0.93	0.94	0.95	0.96	0.98	0.98	0.98	0.98
3			ID	0.90	0.90	0.87	0.89	0.89	0.79	0.90	0.95	0.94	0.94	0.90	0.94	0.92	0.93	0.94	0.94	0.96	0.98	0.98	0.98	0.98
4				ID	0.92	0.94	0.98	0.98	0.80	0.89	0.92	0.91	0.91	0.91	0.91	0.90	0.90	0.91	0.91	0.90	0.89	0.89	0.89	0.89
5					ID	0.90	0.90	0.91	0.81	0.90	0.92	0.92	0.92	0.92	0.92	0.91	0.92	0.92	0.92	0.92	0.90	0.90	0.90	0.90
6						ID	0.93	0.94	0.78	0.88	0.90	0.90	0.90	0.91	0.90	0.90	0.90	0.90	0.90	0.89	0.87	0.87	0.87	0.87
7							ID	0.97	0.79	0.90	0.91	0.90	0.90	0.90	0.90	0.90	0.90	0.91	0.91	0.90	0.89	0.89	0.89	0.89
8								ID	0.79	0.88	0.91	0.90	0.90	0.90	0.91	0.90	0.90	0.91	0.91	0.90	0.89	0.89	0.89	0.89
9									ID	0.80	0.81	0.81	0.80	0.79	0.80	0.79	0.80	0.80	0.81	0.81	0.79	0.79	0.79	0.79
10										ID	0.92	0.91	0.91	0.90	0.91	0.90	0.91	0.92	0.92	0.91	0.90	0.90	0.89	0.89
11											ID	0.99	0.99	0.92	0.99	0.97	0.98	0.99	1.00	0.96	0.95	0.95	0.94	0.94
12												ID	0.99	0.92	0.99	0.97	0.97	0.99	0.99	0.95	0.94	0.94	0.94	0.94
13													ID	0.92	0.99	0.96	0.97	0.99	0.99	0.95	0.94	0.94	0.94	0.94
14														ID	0.92	0.92	0.92	0.93	0.92	0.92	0.90	0.90	0.89	0.89
15															ID	0.97	0.98	0.99	0.99	0.96	0.94	0.94	0.94	0.94
16																ID	0.99	0.96	0.97	0.94	0.92	0.92	0.92	0.92
17																	ID	0.97	0.98	0.95	0.93	0.93	0.93	0.93
18																		ID	0.99	0.95	0.94	0.94	0.94	0.94
19																			ID	0.95	0.94	0.94	0.94	0.94
20																				ID	0.96	0.96	0.96	0.96
21																					ID	1.00	1.00	1.00
22																						ID	1.00	1.00
23																							ID	1.00
24																								ID

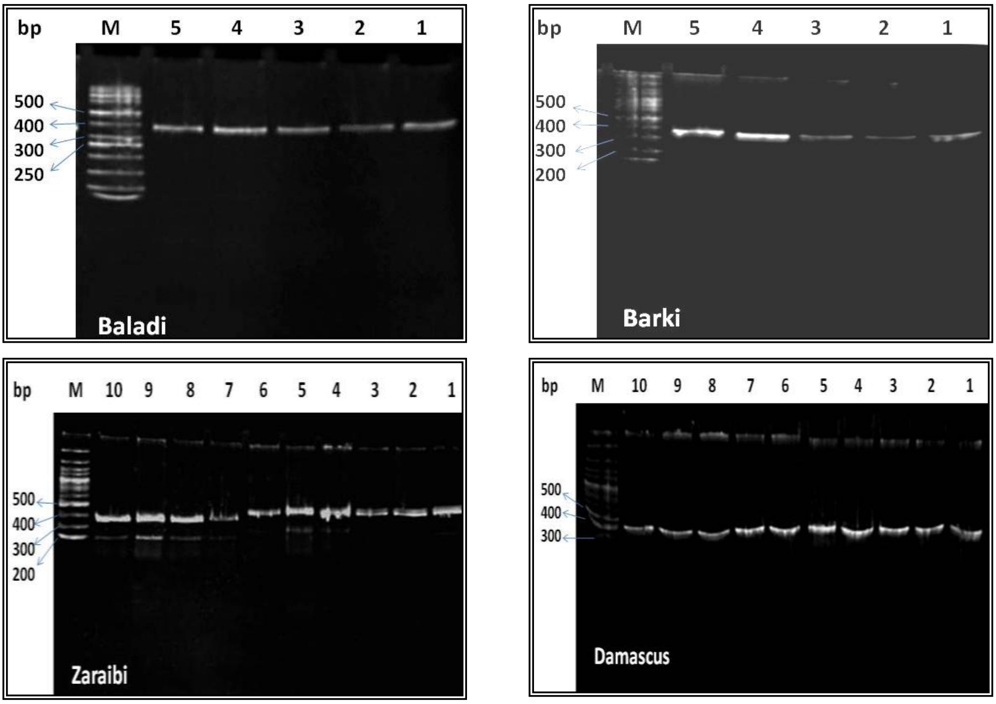
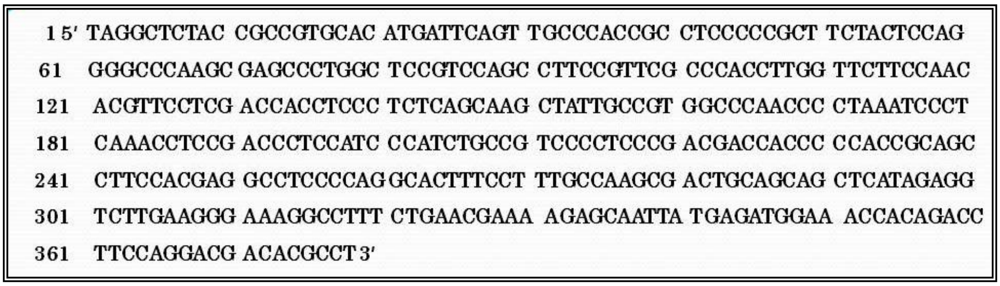


Fig. (1): PCR product for the *Acr* gene, exon 5 fragments of individual samples for (a) Baladi, (b) Barki, (c) Zaraibi and Damascus (d) breeds.



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61   GGGCCCAAGC GAGCCCTGGC TCCGTCCAGC CTTCCGTTCG CCCACCTTGG TTCTTCCAAC
121  ACGTTCCTCG ACCACCTCCC TCTCAGCAAG CTATTGCCGT GGCCCAACCC CTAAATCCCT
181  CAAACCTCCG ACCCTCCATC CCATCTGCCG TCCCCTCCCG ACGACCACCC CCACCGCAGC
241  CTTCCACGAG GCCTCCCCAG GCACTTTCCT TTGCCAAGCG ACTGCAGCAG CTCATAGAGG
301  TCTTGAAGGG AAAGGCCTTT CTGAACGAAA AGAGCAATTA TGAGATGGAA ACCACAGACC
361  TTCCAGGACG ACACGCCT 3'
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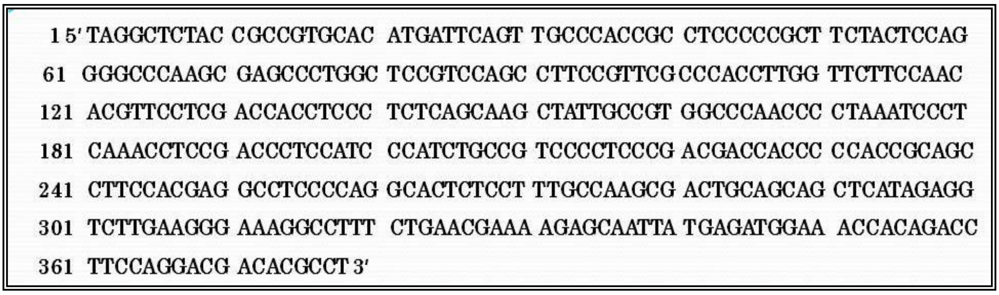
Fig. (2): Nucleotides sequence of *Acr* gene, exon 5 in Baladi breed.



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121  ACGTTCCTCG ACCACCTCCC TCTCAGCAAG CTATTGCCGT GGCCCAACCC CTAAATCCCT
181  CAAACCTCCG ACCCTCCATC CCATCTGCCG TCCCCTCCCG ACGACCACCC CCACCGCAGC
241  CTTCCACGAG GCCTCCCCAG GCACTCTCCT TTGCCAAGCG ACTGCAGCAG CTCATAGAGG
301  TCTTGAAGGG AAAGGCCTTT CTGAACGAAA AGAGCAATTA TGAGATGGAA ACCACAGACC
361  TTCCAGGACG ACACGCCT 3'
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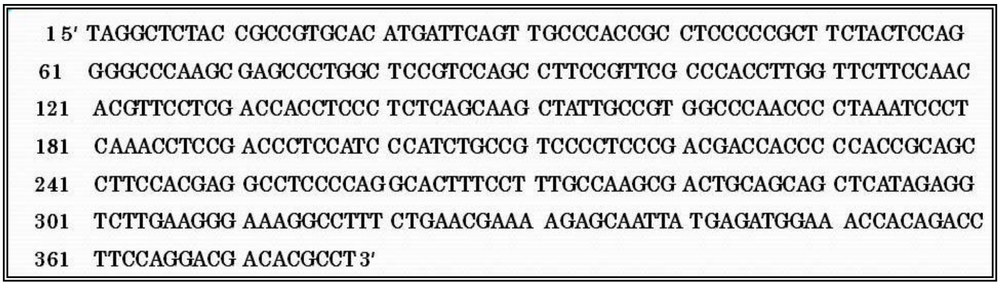
Fig. (3): Nucleotides sequence of *Acr* gene, exon 5 in Barki breed.



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61   GGGCCCAAGC GAGCCCTGGC TCCGTCCAGC CTTCCGTTCG CCCACCTTGG TTCTTCCAAC
121  ACGTTCCTCG ACCACCTCCC TCTCAGCAAG CTATTGCCGT GGCCCAACCC CTAAATCCCT
181  CAAACCTCCG ACCCTCCATC CCATCTGCCG TCCCCTCCCG ACGACCACCC CCACCGCAGC
241  CTTCCACGAG GCCTCCCCAGGCACTTTCCT TTGCCAAGCG ACTGCAGCAG CTCATAGAGG
301  TCTTGAAGGG AAAGGCCTTT CTGAACGAAA AGAGCAATTA TGAGATGGAA ACCACAGACC
361  TTCCAGGACG ACACGCCT 3′
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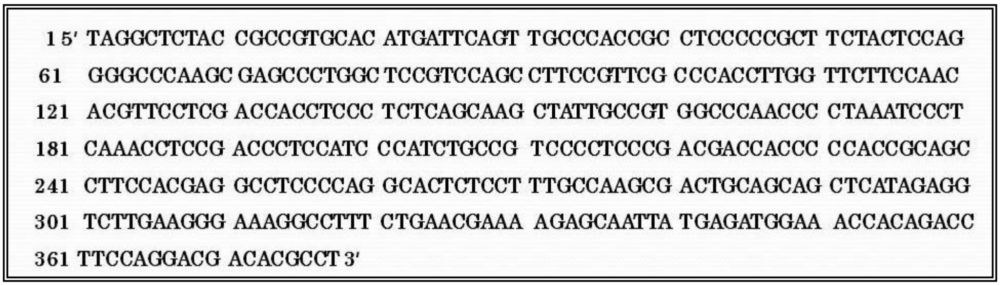
Fig. (4): Nucleotides sequence of *Acr* gene, exon 5 in Zaraibi breed.



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61   GGGCCCAAGC GAGCCCTGGC TCCGTCCAGC CTTCCGTTCG CCCACCTTGG TTCTTCCAAC
121  ACGTTCCTCG ACCACCTCCC TCTCAGCAAG CTATTGCCGT GGCCCAACCC CTAAATCCCT
181  CAAACCTCCG ACCCTCCATC CCATCTGCCG TCCCCTCCCG ACGACCACCC CCACCGCAGC
241  CTTCCACGAG GCCTCCCCAG GCACTCTCCT TTGCCAAGCG ACTGCAGCAG CTCATAGAGG
301  TCTTGAAGGG AAAGGCCTTT CTGAACGAAA AGAGCAATTA TGAGATGGAA ACCACAGACC
361 TTCCAGGACG ACACGCCT 3′
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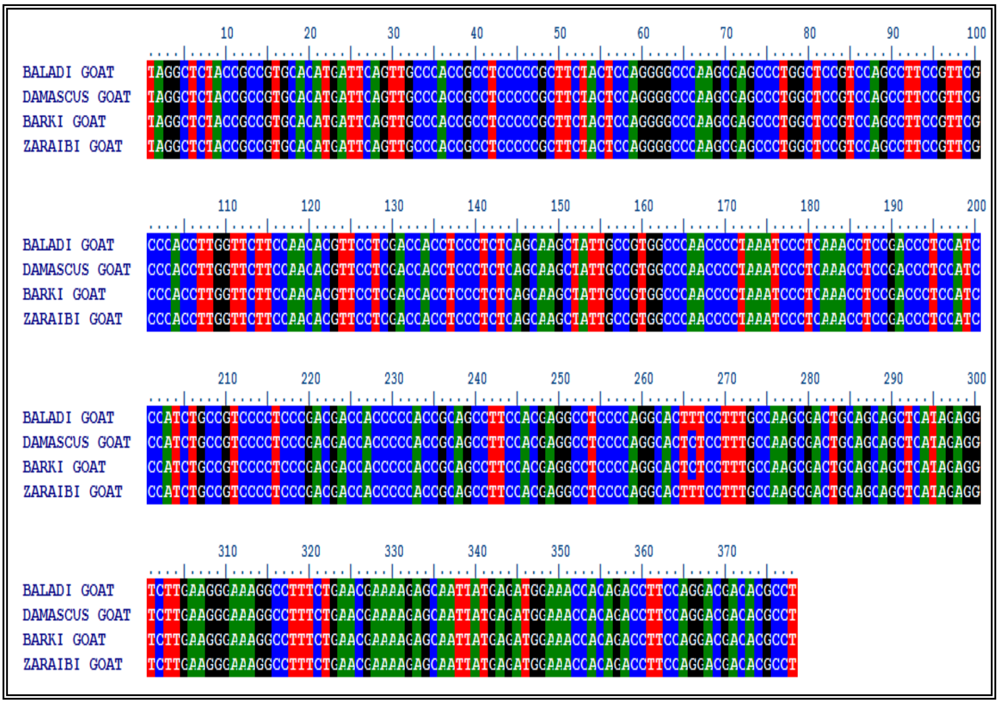
Fig. (5): Nucleotides sequence of *Acr* gene, exon 5 in Damascus breed.



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                       10        20        30        40        50        60        70        80        90       100
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BALADI GOAT    TAGGCTCTACCGCCGTGCACATGATTCAGTTGCCCACCGCCTCCCCCGCTTCTACTCCAGGGGCCCAAGCGAGCCCTGGCTCCGTCCAGCCTTCCGTTCG
DAMASCUS GOAT  TAGGCTCTACCGCCGTGCACATGATTCAGTTGCCCACCGCCTCCCCCGCTTCTACTCCAGGGGCCCAAGCGAGCCCTGGCTCCGTCCAGCCTTCCGTTCG
BARKI GOAT     TAGGCTCTACCGCCGTGCACATGATTCAGTTGCCCACCGCCTCCCCCGCTTCTACTCCAGGGGCCCAAGCGAGCCCTGGCTCCGTCCAGCCTTCCGTTCG
ZARAIBI GOAT   TAGGCTCTACCGCCGTGCACATGATTCAGTTGCCCACCGCCTCCCCCGCTTCTACTCCAGGGGCCCAAGCGAGCCCTGGCTCCGTCCAGCCTTCCGTTCG

                      110       120       130       140       150       160       170       180       190       200
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BALADI GOAT    CCCACCTTGGTTCTTCCAACACGTTCCTCGACCACCTCCCTCTCAGCAAGCTATTGCCGTGGCCCAACCCCTAAATCCCTCAAACCTCCGACCCTCCATC
DAMASCUS GOAT  CCCACCTTGGTTCTTCCAACACGTTCCTCGACCACCTCCCTCTCAGCAAGCTATTGCCGTGGCCCAACCCCTAAATCCCTCAAACCTCCGACCCTCCATC
BARKI GOAT     CCCACCTTGGTTCTTCCAACACGTTCCTCGACCACCTCCCTCTCAGCAAGCTATTGCCGTGGCCCAACCCCTAAATCCCTCAAACCTCCGACCCTCCATC
ZARAIBI GOAT   CCCACCTTGGTTCTTCCAACACGTTCCTCGACCACCTCCCTCTCAGCAAGCTATTGCCGTGGCCCAACCCCTAAATCCCTCAAACCTCCGACCCTCCATC

                      210       220       230       240       250       260       270       280       290       300
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BALADI GOAT    CCATCTGCCGTCCCCTCCCGACGACCACCCCCACCGCAGCCTTCCACGAGGCCTCCCCAGGCACTTTCCTTTGCCAAGCGACTGCAGCAGCTCATAGAGG
DAMASCUS GOAT  CCATCTGCCGTCCCCTCCCGACGACCACCCCCACCGCAGCCTTCCACGAGGCCTCCCCAGGCACTCTCCTTTGCCAAGCGACTGCAGCAGCTCATAGAGG
BARKI GOAT     CCATCTGCCGTCCCCTCCCGACGACCACCCCCACCGCAGCCTTCCACGAGGCCTCCCCAGGCACTCTCCTTTGCCAAGCGACTGCAGCAGCTCATAGAGG
ZARAIBI GOAT   CCATCTGCCGTCCCCTCCCGACGACCACCCCCACCGCAGCCTTCCACGAGGCCTCCCCAGGCACTTTCCTTTGCCAAGCGACTGCAGCAGCTCATAGAGG

                      310       320       330       340       350       360       370
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
BALADI GOAT    TCTTGAAGGGAAAGGCCTTTCTGAACGAAAAGAGCAATTATGAGATGGAAACCACAGACCTTCCAGGACGACACGCCT
DAMASCUS GOAT  TCTTGAAGGGAAAGGCCTTTCTGAACGAAAAGAGCAATTATGAGATGGAAACCACAGACCTTCCAGGACGACACGCCT
BARKI GOAT     TCTTGAAGGGAAAGGCCTTTCTGAACGAAAAGAGCAATTATGAGATGGAAACCACAGACCTTCCAGGACGACACGCCT
ZARAIBI GOAT   TCTTGAAGGGAAAGGCCTTTCTGAACGAAAAGAGCAATTATGAGATGGAAACCACAGACCTTCCAGGACGACACGCCT
```

Fig. (6): Multiple alignment of the nucleotides sequence of the 378 bp for the *Acr* gene, exon 5 in the four studied goat breeds.

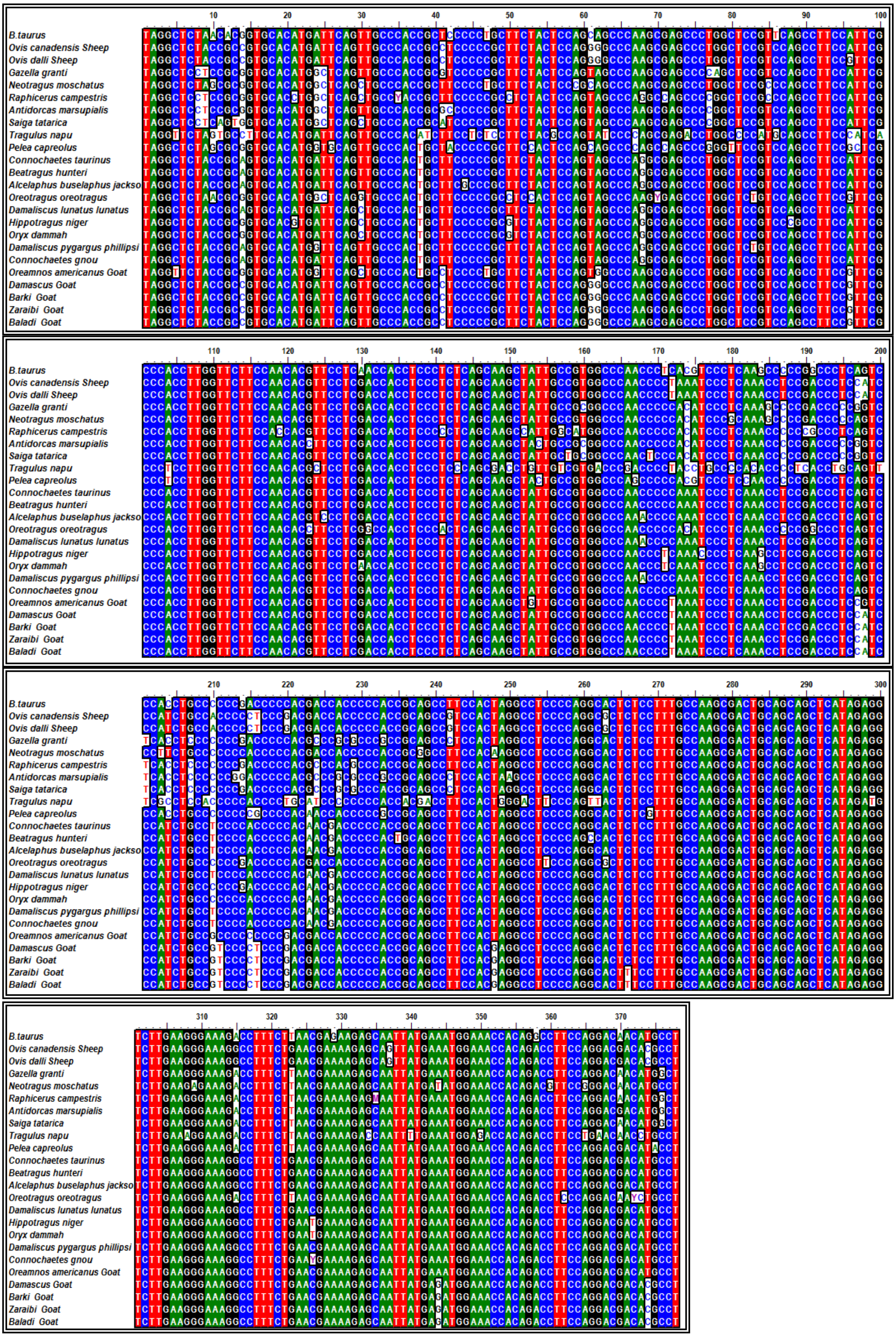

Fig. (7): Multiple alignment of the nucleotides sequence of the obtained 378 bp for the *Acr* gene, exon 5 with the other 20 origins.

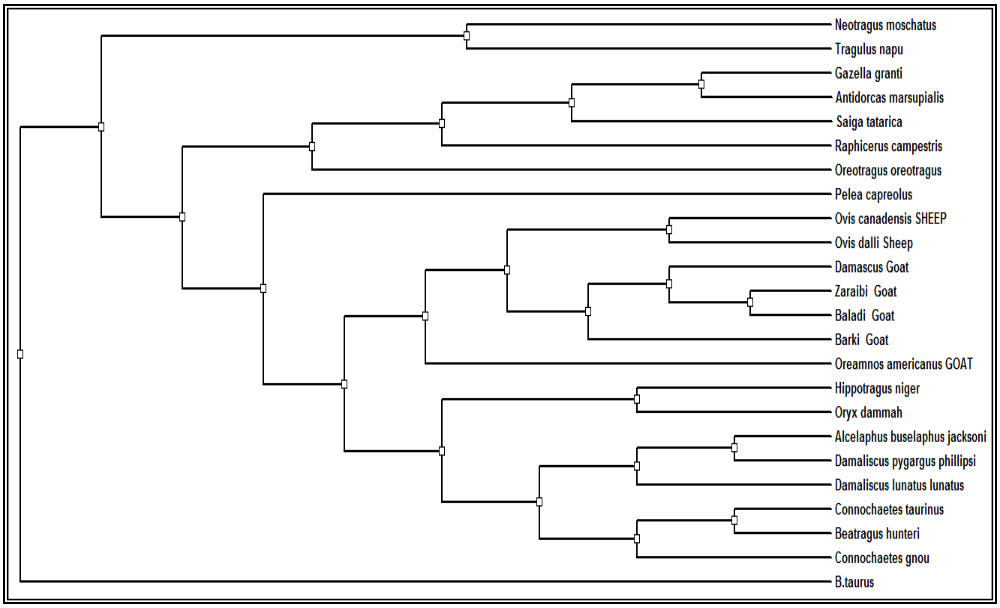

Fig. (8): Genetic relationship among the four studied breeds and other 20 origins based on *Acr* gene, exon 5 alignment.