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Genetic Diversity among Seven Goat Breeds assessed by Inter Simple Sequence Repeats (ISSR).

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ABSTRACT

DNA of the seven goat breeds germplasm (Balady, Pakistani, Chady, Hejazy, Damascus, Kubbrosy and Jumnapari) live in Libya were extracted and subjected to molecular analysis using Inter simple sequence repeats (ISSR). Ten ISSR specific primers were surveyed and five out of the used primers only produced scorable banding patterns.

A total numbers of 56 bands were produced overall the five ISSR primers which produced a scorable banding patterns. Out of the produced bands, 12 fragments were monomorphic and 44 amplified fragments were polymorphic in the seven goats' germplasm. The Percentage values of polymorphic bands were 100%, 60%, 54.54%, 91.67% and 100% for HB10 , HB12, HB13, 814 and 17899A primers, respectively. Out of the 44 polymorphic fragments there were 13 fragments considered positive and 3 considered negative germplasm-specific marker.

It can conclude from this study that the used ISSR primers produced polymorphic loci and seemed to be useful for the population genetic studies of goat's breeds.

INTRODUCTION

Genetic diversity plays an important role in survival and adaptability of species. It is affected by the small size of population and level of intervene of human. With little variation of gene, the reproduction efficiency decrease and some types of diseases increase (Paetkau et al., 2008).

In recent years, molecular methods are modern tools, which assist in a better understanding of genetic diversity. Inter-simple sequence repeats method (ISSRs) consider one from these methods. ISSR are regions in the genome flanked by microsatellite sequences. PCR amplification of these regions using a single primer yields multiple amplification products that can used as a dominant multi locus marker system for the study of genetic variation in various organisms. (Ng and Tan, 2015).

ISSR marker is similar to random amplification of polymorphic DNA (RAPD) and can used without knowing the sequence information for genomic DNA (Zietkiewicz et al., 1994). ISSR markers are easy to use, methodologically less demanding, good reproducibility, reasonable cost and only a small amount of DNA is needed compared to other dominant markers, permitting its use for genetic studies of population of different species (Dogan et al., 2007; Hakki et al., 2010; Wang et al., 2008).

The aim of the study was to use ISSR markers to estimate genetic diversity among seven goat breeds.

MATERIALS AND METHODS

Photos of goat breeds used in current study to detect the genetic diversity among them by using ISSR are shown next. Inter simple sequences repeats (ISSR)

The basic procedure to conduct an ISSR genotyping experiment is simple: PCR, using an ISSR primer, with genomic DNA (gDNA) as its template; use of agarose or polyacrylamide gel electrophoresis of PCR amplification products; scoring of ISSR bands; and data analysis.

DNA extraction

Animals were randomly selected from 7 goat breeds, Blood samples were collected into vacutainers with EDTA anticoagulant. Samples kept at -20°C until use. Total DNA extracted from one ml of blood sample using a Bioflux Kit (from China) as described in the kit manual.

(1) Balady

(2) Chady

(3) Pakistani

(4) Jumnapari

(5) Damascus

(6) Hejazy

(7) Kubbrosy

Photos (1): The morphological of goat's breeds under Study.

PCR conditions and electrophoresis

Five ISSR primers were screened in order to select those could amplify the extracted DNA (Table 1). The PCR amplification aimed to the selection of ISSR primers giving few discrete, large bands using several annealing temperatures. A total volume of 25 µL of a solution containing 2.5 µl of dNTPs (8 mM mix), 0.2 µl of Taq DNA polymerase (5 U/ µl), 2.5 µl of 10 X buffer with 15 mM MgCl2, 1µl of Primer (10mM), 1µl of Template DNA (10-50 ng/ µl) , 3 µl of Mg Cl_2 , 16.3 µl of H_2O (dd).

PCR cycling conditions were 94ºC for 4 minutes, 40 cycles of (denaturing at 94ºC for 1.30 mins, 1.30 mins at annealing temperature and elongation at 72 ºC for 2 mins) and final elongation at 72º C for 7 mins.

Table (1): List of ISSR Primers Used

Primer	Sequence
HB10	$(GA)_6$ CC
HB12	$(CAC)_3$GC
HB13	(GAG)3GC
814	$(CT)_8$TG
17899A	$(CA)_6$ AG

All PCR reactions performed with thermocycler MJ min I PTC (Bio Rad). The result of each amplification reaction analyzed on 2% agarose gel in 1xTBE buffer and run at 100 volt for 1 hour. Bands detected on UV-Transilluminator and photographed by gel documentation system (Biometra Bio Doc

Analyzer - 2000). The polymerase chain reaction products were resolved on T.A.E. (Tris-acetate-EDTA), buffer used as both a running buffer and in agarose gel.

There is some concepts must understood when we tabulate ISSR band-counts, commonly reported in studies use ISSR markers: a 'band' scored in an ISSR experiment can also be termed as a 'locus'. Regardless whether a series of ISSR bands are generated from the same PCR reaction using the same ISSR primer, each ISSR band is separately considered as one locus, and represent one data point in any analysis; "total number of bands" is the total number of different ISSR band sizes observed across all samples in a study and "number of polymorphic bands" is the number of ISSR bands that show variation, i.e. the bands present for some samples and absent for the others.

The band presents in all goats' germplasm under investigation while absent in a particular germplasm is considered negative germplasm specific marker (NGSM). On contrary, the band absent in all goats' germplasm under investigation while present in a particular germplasm considered as a positive germplasm specific marker (PGSM).

Finally, bands recorded into the binary symbols "1" for band presence, and "0" for band absence, for subsequent analyses. Percentage of polymorphic bands are represented by the formula:

$$\% \text{ of polymorphic bands} = \frac{\text{No of polymorphic bands}}{\text{Total No of bands}} \times 100$$

RESULTS

DNA of the seven goats' germplasm under investigation were extracted and subjected to molecular analysis using ISSR with ten inter simple sequences repeats specific primers, five out of the used primers only produced scorable banding patterns.

ISSR considered as a co-dominant marker. It depends on PCR to amplify DNA fragments located in tentative simple sequence repeats or microsatellite which could be closely related to particular functional gene.

A total numbers of 56 bands were produced overall the five ISSR primers which produced a scorable banding patterns. Out of the produced bands, 12 fragments were monomorphic in the seven goats' germplasm and 44 amplified fragments were polymorphic.

Out of the 44 polymorphic fragments there were 13 fragments considered positive germplasm-specific markers (PGSM) and 3 considered negative germplasm-specific marker (NGSM).

The results of HB10 primer shown in Table (2) and Figure (1). The primer produced six bands with the molecular sizes ranged from 300 to 720bp. All bands produced by this primer were polymorphic and no monomorphic band was found**.** In other word, number and percentage of monomorphic fragments equaled zero (no similarity) and 6 (100%) for polymorphic (bands show variation).

Furthermore, the results showed that bands had molecular size 720 bp for Chady germplasm and 580 bp for Damascus goat. Owing to these bands were presented in a particular germplasm, it considered as a PGSM.

The results of HB12 primers are illustrated in Table (3) and Figure (2). The primers produced 15 bands with molecular sizes ranged from 250 to 1550bp. Number and percentage of monomorphic fragments equal 6 (40%) (bands show similarity) and 9 (60%) for polymorphic (bands show variation) according to this primer. In addition, the results indicate that three bands with molecular sizes 410, 350 and 270 bp were presented only in Pakistani germplasm and a band with molecular size 390 bp was detected in Jumnapari germplasm, thus these bands considered as PGSM.

On the other hand, bands with molecular sizes 480 bp and 250 bp were detected in Jumnapari and in Pakistani goat's for the sake of these bands considered as NGSM

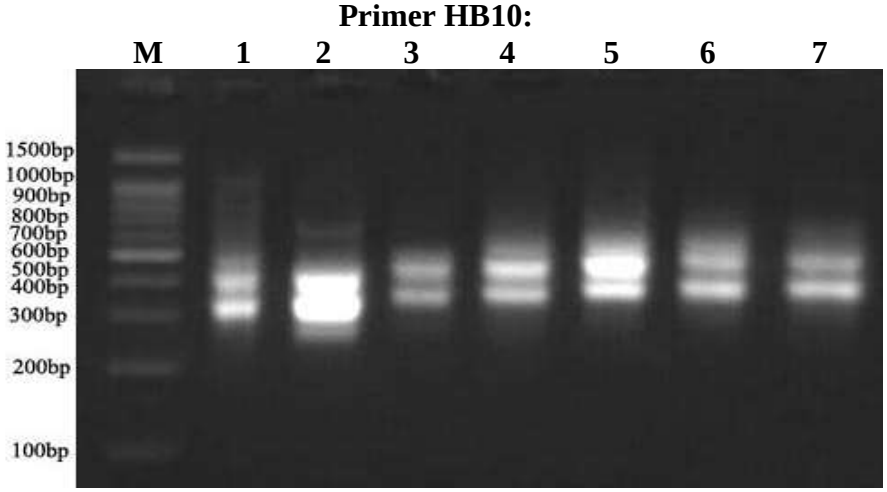

Fig. (1): Banding patterns of the seven goat's under investigation, using HB10 primer

Table (2): The molecular size, numbers of bands scored and percent. monomorphic & polymorphic bands using HB10 primer.

Bp	Pakistani	chady	Hejazy	Damascus	Balady	kubbrossy	Jumnapari
720	0*	1	0	0	0	0	0
580	0	0	0	1	0	0	0
460	0	0	1	1	1	1	1
400	1	1	0	0	0	0	0
370	0	0	1	1	1	1	1
300	1	1	0	0	0	0	0
No. and Percent. of monomorphic fragments (0)				**No. and Percent. of polymorphic fragments 6 (100%)**			

*** 1= presence and 0 = absence**

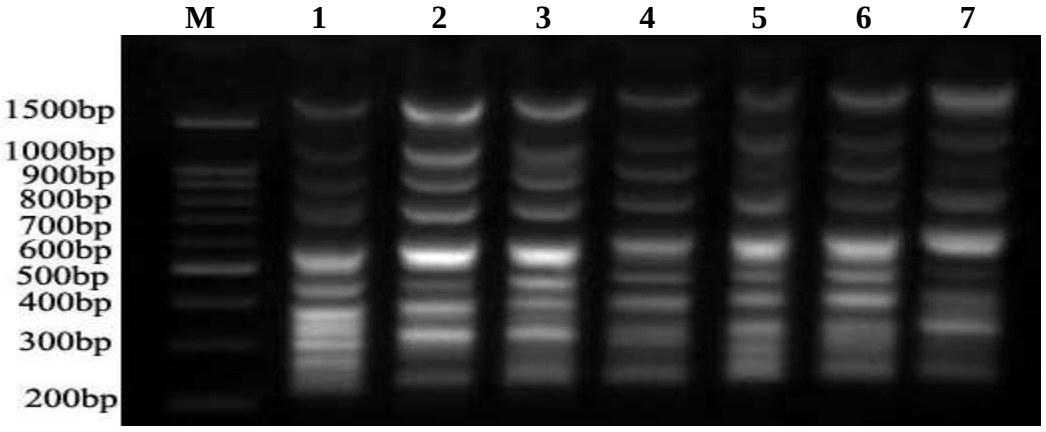

Figure (2): Banding patterns of the seven goats' under investigation using HB12 primer.

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Table (3): The molecular size, numbers of bands scored and percent. monomorphic & polymorphic bands using primer HB12 primer.

Bp	Pakistani	Chady	Hejazy	Damascus	Balady	Kubbrossy	Jumnapari
1550	1	1	1	1	1	1	1
1000	1	1	1	1	1	1	1
880	1	1	1	1	1	1	1
770	1	1	1	1	1	1	1
560	1	1	1	1	1	1	1
480	1	1	1	1	1	1	0
440	1	1	1	1	1	1	1
410	1	0	0	0	0	0	0
390	0	0	0	0	0	0	1
370	0	1	1	1	1	0	0
350	1	0	0	0	0	0	0
330	0	1	1	1	1	0	1
300	0	0	0	1	0	0	0
270	1	0	0	0	0	0	0
250	0	1	1	1	1	1	1
No. and Percent. of monomorphic fragments 6(40.0%)				**No. and Percent . of polymorphic fragments 9 (60.0%)**			

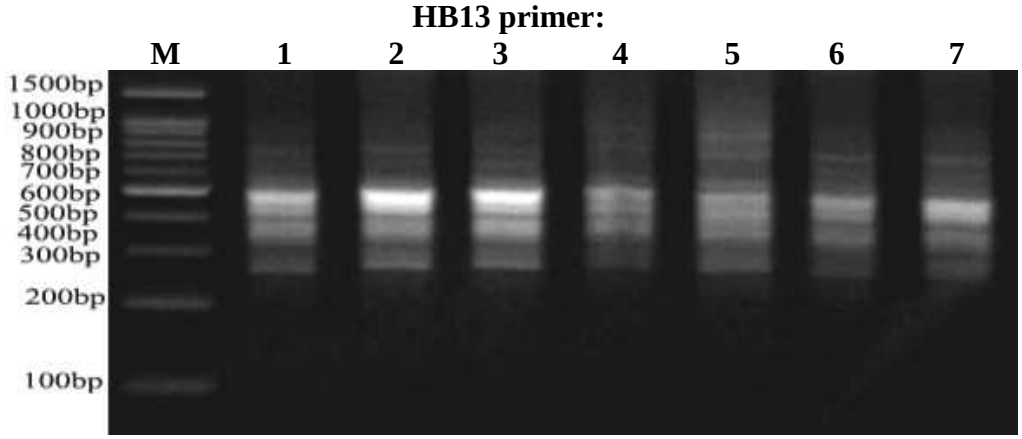

Figure (3): Banding patterns of the seven goats' under investigation using HB13 primer

The results of HB13 primer are shown in Table (4) and Figure (3). The primers produced eleven bands with molecular sizes ranged from 260 to 980 bp. Number and percent. of monomorphic fragments equal 5(45.45%) and 6 (54.54%) for polymorphic in accordance with this primer. Beside that, it found a PGSM with molecular size 500 bp and a NGSM with molecular size 470 bp in Damascus germplasm.

Table (4): The molecular size, numbers of bands scored and percent. monomorphic & polymorphic bands using HB13 primer.

Bp	Pakistani	Chady	Hejazy	Damascus	Balady	Kubbrossy	Jumnapari
980	0	0	0	1	1	0	0
890	1	1	1	1	1	1	1
820	1	1	1	0	0	1	1
740	1	1	1	1	1	1	1
650	1	1	1	1	1	1	1
580	1	1	1	1	1	1	1
500	0	0	0	1	0	0	0
470	1	1	1	0	1	1	1
410	1	1	1	1	0	0	1
370	0	0	0	0	1	1	0
260	1	1	1	1	1	1	1

No. and Percent. of monomorphic fragments	**No. and Percent . of polymorphic fragments**
5 (45.45 %)	**6 (54.54%)**

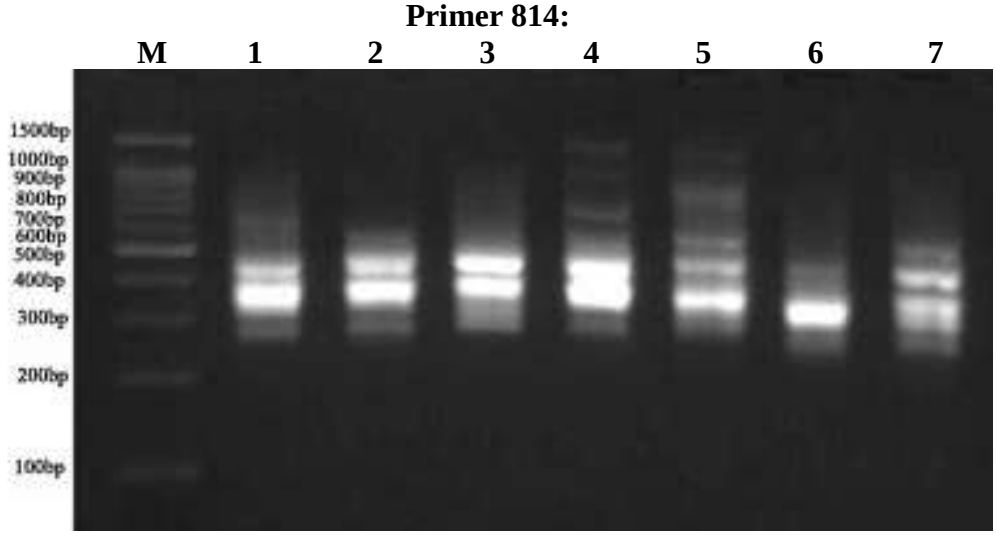

Figure (4): Banding patterns of the seven goats' under investigation using 814 primer.

The results of 814 primers are presented in Table (5) and Figure (4). The primers produced 12 bands with molecular sizes ranged from 240 to 1210 bp. Number and percent. of monomorphic fragments equal 1(8.33%) and 11 (91.67%) for polymorphic in accordance with this primer.

Moreover, the results showed that three PGSM were produced with lengths 1210, 1000 and 340 bp in Damascus and two more PGSM bands.

Table (5): The molecular size, numbers of bands scored and percent. monomorphic & polymorphic bands using primer 814 primer.

Bp	Pakistani	Chady	Hejazy	Damascus	Balady	Kubbrossy	Jumnapari
1210	0	0	0	1	0	0	0
1180	0	0	0	0	1	0	0
1000	0	0	0	1	0	0	0
790	0	0	0	0	1	0	0
740	1	0	0	1	0	0	0
590	0	1	0	0	1	0	1
460	1	1	1	1	1	1	1
370	1	1	1	0	0	0	1
340	0	0	0	1	0	0	0
310	1	0	0	0	0	1	0
385	0	1	0	0	0	0	1
260	1	1	0	0	0	0	0
No. and Percent. of monomorphic fragments 1(8.33%)				**No. and Percent. of polymorphic fragments 11 (91.67%)**			

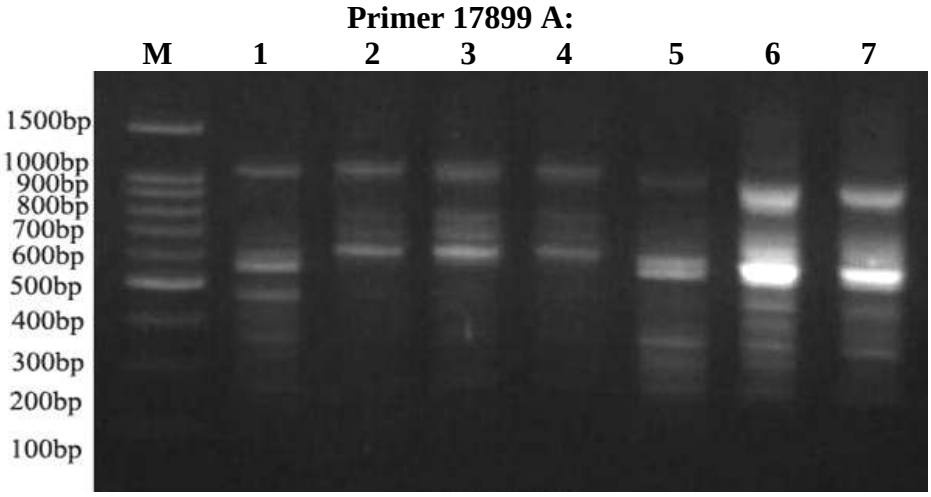

Fig. (5): Banding patterns of the seven goat's under investigation using 17899A primer.

were found with lengths 1180 and 790 bp in Balady goat's germplasm The results of 17899A primers are indicated in Table (6) and Figure (5). The primers produced 12 bands with molecular sizes ranged from 210 to 1190bp. Number and percent. of monomorphic fragments equal zero (no similarity) and 6 (100%) for polymorphic (bands show variation).

DISCUSSION

Biological diversity is commonly a recognized value in natural resources management where managing the genetic diversity within a species is valuable. Losing genetic diversity is considered the main reason of high level of breeds uniformity and will increase frequency of genetical defects and negatively affects fertility. Thus, maintaining the genetic diversity of livestock species is one of the paramount interests for agricultural

Table (6): Screening of ISSR banding patterns in the seven goats' germplasm under investigation, using primer 17899A where (1) presence, (0) absence.

Bp	Pakistani	chady	Hejazy	Damascus	Balady	kubbrossy	Jumnapari
1190	0	0	0	0	0	1	1
1100	1	1	1	1	1	0	0
890	0	0	0	0	0	1	1
800	0	1	1	1	0	0	0
750	0	1	1	1	0	0	0
700	0	1	1	1	0	0	0
580	1	0	0	1	0	1	0
550	0	0	0	0	1	1	1
490	1	0	0	0	1	1	1
360	1	0	0	0	0	1	1
280	0	0	0	0	1	1	0
210	0	0	0	0	1	1	0
No. and Percent. of monomorphic fragments (0)				**No. and Percent. of polymorphic fragments 12(100%)**			

policies and serious steps are necessary to be taken in order to protect the stocks of local animals, which are in danger of extinction (Askari et al., 2011).

Each breed (5 samples clustered together in one separate clade) explained the fact that each sample belongs to the same breed and there was a high similarity between individuals (Khaldi et al., 2010). This results suggest high percentage of homogeneity within each breed and high level of genetic variability among studied breeds. The found interbreed variability might due to a difference in the population, individual variations and/or might due to genetic stratification (Ziv, and Burchard 2003).

Moradi et al. (2014) evaluated the genetic diversity of Iranian Markhoz goat and its color types using ISSR markers. Four primers were designed and two of them selected based on their number of bands and polymorphic properties. These primers generated 28 amplification products, from which 23 bands were polymorphic, revealing 82.14% polymorphism. Although the primers were still different and consider as species differences, the results showed that the genetic diversity of Markhoz goat was less than Kermani sheep and higher than Iranian Holstein cattle. This may suggest using ISSR markers as a well candidate tool for these analyses for these animals and the combination of ISSR and other markers will eventually lead to produce a better description of the variation in stocks of local animals, which are in danger of extinction.

In current study, the variation of size and number of amplified fragments among goat breeds could be a result of nucleotide changes at the primer annealing site or due to deletion or addition between two priming sites. Moreover, these variations illustrate the different pattern of amplification of primers. Furthermore, it suggests the genetic heterogeneity between and within breeds (Sharma et al., 2001 and El-tarras et al., 2015).

While results of ISSR genotyping is reproducible within a study using the same equipment and protocol, it recommend caution when comparing band-scoring results across different researches. Even when, for example, two separate studies are on the same species using the same primers, the ISSR banding patterns may vary considerably (Ng and Szmidt 2014). The cause for this is unknown, but it could due to the effect of using different reagents (each manufacturer possibly have their own reagent concoction, including additives) and settings during PCR and/or the different scoring criteria adopted by different researchers. These

inconsistencies make the reporting of experimental protocol very important in studies using ISSR markers. To demonstrate the robustness of studies involving ISSR markers, we have adapted recommendations by Crawford et al. (2012) and urge researchers to explicitly report the following in their manuscripts. Steps taken throughout the ISSR experiment is; names and sequences of the ISSR primers used, the PCR reaction protocol associated with each primer; standards/criteria used to ensure reliability of genotyping and scoring of bands, i.e. by selecting only loci that are clear, unambiguous, reproducible, shown in replicated experiments and number and proportion of samples used in the replicated experiment.

CONCLUSION

The present study indicate that ISSR marker can successfully employed to assess the breed level of polymorphism and genetic diversity.

From our knowledge, this work consider the first application of this method to compare the seven goat breeds studied. The results of this research will provide evidence for the reliability and usefulness of ISSR markers to estimate genetic diversity among different goat breeds.

For economic and accurate reasons we must make comparison among molecular techniques such as RAPD and ISSR. In addition, different biochemical techniques such as protein electrophoresis and isozymes that could use for assessment of genetic diversity among different species and breeds.

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