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Full Length Research Paper

Genetic Variation in Captive Herd of Arabian Oryx Using RAPD and ISSR Markers

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According to the international taxonomy of IUCN, the Arabian Oryx (*Oryx leucoryx*) is considered to be in endangered and included among the animals that may be extinct. Saudi Arabia began an important project of captive breeding of this species in 1986 at the National Wildlife Research Center (NWRC). Therefore, the current captive population of Saudi Arabian Oryx at NWRC may suffer from inbreeding depression due to the population of small sizes. The genetic variation among 10 Arabian Oryx animals was studied using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. The animals were chosen as a representative sample of NWRC current herd. The objectives of this study were: (1) Establishment of RAPD and ISSR marker sets for the genetic characterization of Saudi Arabian Oryx and (2) application of the RAPD and ISSR markers for the identification and estimation of the genetic variation among the 10 Arabian Oryx animals. The results of the RAPD and ISSR primers used in this study confirmed that there was no sex specificity for any of them. 20 random primers were used for the RAPD experiment that generated 118 fragments with 93.5% polymorphism (110 fragments). Also, 12 ISSR primers were used to detect the polymorphism level among the 10 animals of Arabian Oryx. These primers generated 89 fragments and the number of polymorphic fragment was 65 (71.5%). At the same time, they showed average heterozygosity (variability measurement) of 0.24 among the 89 ISSR loci. The result of the genetic variation of RAPD and ISSR indicated a high level of variation among the 10 genotypes as a representative sample of the animals from the germplasm of NWRC. Finally, it can be recommended that the amount of variations among the NWRC germplasm is enough for selection and breeding improvement without affecting the genetic diversity within the captive herd.

Key words: Arabian Oryx, captive herd, National Wildlife Research Center (NWRC), random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), genetic diversity, Saudi Arabia.

INTRODUCTION

Arabian Oryx (*Oryx leucoryx*) is in the taxonomic phylum *Chordata*, class Mammalia, order Artiodactyla, family Bovidae and subfamily Hippotraginae. This subfamily includes three genus; *Hippotragus*, *Addax* and *Oryx*. The genus Oryx includes three species namely; *Oryx dammah*, *Oryx gazelle* and *Oryx leucoryx*. The last species which is called Arabian Oryx settled in the stoned sandy areas of Arabian Peninsula (Skinner and Chimimba, 2005). However, the Arabian Oryx *(O. leucoryx)* had extinct in the wild during the 1970's due to habitat degradation and over hunting (Henderson, 1974). According to the IUCN red list of threatened species in 2009, the Arabian Oryx species qualifies for the endangered (EN) category under criterion D (less than 250 mature individuals (http://www.iucnredlist.org.) Since captive breeding is known to be effective in the conservation of threatened species, the "World Herd" was established in the United States in 1963 and subsequently, other projects in Arabian countries were initiated. As a result, Arabian Oryx were released in the wild in Jordan in 1978 and in Oman in 1982. Saudi Arabia had begun an important project of captive breeding of this species in 1986 at the National Wildlife Research Center (NWRC) near Taif, Saudi Arabia. Consequently, the Arabian Oryx

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was first released in Saudi Arabia in 1990 into a protected desert area in the west central region (Ostrowski et al., 1998) and later reintroduced into a protected sand dune area (Messochina et al., 2003). Currently, the NWRC captive breeding herd consists of about 113 animals. (http://www.arabian-oryx.gov.sa.).

The current captive population of Saudi Arabian Oryx at NWRC may suffer from inbreeding depression due to the population small size. Inbreeding depression generates offspring with lower fitness than their non-inbred counterparts (Strauss, 2006). Increasing inbreeding depression may cause decrease in the potential rate of genetic diversity (Shobrak, 2007). Thus, knowledge of the variation among individuals is particularly important in captive breeding programs to monitor the impacts of inbreeding on genetic diversity (Montgomery et al., 1998; Frankham et al., 2002). Also, information on genetic diversity is necessary to establish better management plans for the conservation and recovery of the endangered population (Frankham et al., 2002; Russello and Amato, 2007).

Advances in biotechnology offer possibilities of improving, utilizing and conserving the present Arabian Oryx diversity. Among these technologies is the well established polymerase chain reaction (PCR) technology. Many PCR-based markers are very suitable for genetic diversity estimation. One of them is random amplified polymorphic DNA (RAPD) marker, which has many advantages such as; (1) is easy to work with; (2) does not require radioactivity; (3) requires the least amount of DNA; (4) does not need any sequence information and (5) it is less costly and time effective for studies involving small sample sizes. Several studies have employed RAPD markers for various applications including the estimation of genetic diversity among cattle herds (Alves, et al., 2005), horses (Alves et al., 2007) and goat (Gaali and Satti, 2009).

Also, since 1994, a new molecular marker technique called inter simple sequence repeat (ISSR) has been available (Zietkiewicz et al., 1994). ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite. Each band corresponds to a DNA sequence delimited by two inverted microsatellites. Like RAPDs, ISSRs markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers. ISSR primers could be successfully used for the detection of new genomic loci and applied in a new way for genomic mapping, fingerprinting, gene tagging and genetic diversity studies. Different studies have been performed on animals, using ISSR markers (Triapitsyna and Glazko, 2005; Bannikova, 2004; Vaulin and Zakharov, 2008; Glazko et al., 1999; Lovenko, 2002; Gorodnaya and Glazko, 2006; Chatterjee and Mohandas, 2003). Furthermore, many studies have shown that, the approach can be used as a useful tool for the genetic diversity monitoring in different populations (or breeds) of animals (Ahani Azari et al., 2007; Kol and Lazebny, 2006).

Currently, Saudi Arabia's conservation programs for the protection of large areas within the former range of the Arabian Oryx and the captive breeding of Oryx at the NWRC have together enabled the restoration of the species in the Kingdom. The first reintroduction took place in the fenced Mahazat as-Sayd protected area in 1990 and in 1995 a free-ranging population of Oryx was established in the unfenced 'Uruq Bani Ma'arid protected area in the "Empty Quarter". The 'Uruq Bani Ma'arid protected area is the only place in the world where a free-ranging Oryx population existed and Mahazat as-Sayd protected area harbored more than 500 Oryx (Strauss, 2006). However, little is known about the genetic variability in this relict species, which is obviously essential for the genetic management of the captive animals and making of the releasing program. Therefore, in this study, RAPD and ISSR markers based on the polymerase chain reaction (PCR), was developed to establish the genetic variation within the captive herd from NWRC. The specific objectives of this research were: (1) Establishment of RAPD and ISSR marker sets for the genetic characterization of Saudi Arabian Oryx and (2) application of the RAPD and ISSR markers for the identification and estimation of the genetic variation among 10 Arabian Oryx animals as a representative sample of the captive herd from NWRC.

Finally, this type of study would help in the future selection which should be based on molecular markers patterns since it is partially available in order to preserve as much variation as possible and to limit any type of inbreeding.

MATERIALS AND METHODS

Samples

Blood samples were collected from Arabian Oryx, National Wildlife Research Center, Taif, Saudi Arabia. The samples included males and females (Table 1). The samples were obtained from 10 captive Arabian Oryx (*O. leucoryx*).

Blood samples collection

Animals were manually anesthetized and darted with rifle or blowpipe depending on the distance from the animal. Blood was collected from the jugular vein with an 18 gauge needle into 10 ml vacutainer tubes (heparin). Samples were refrigerated until examined.

Random amplified polymorphic DNA (RAPD) technique

DNA extraction

DNA was extracted from the whole blood according to the method of Alves et al. (2005). The DNA isolated from the blood samples were collected in vacuum tubes containing heparin. The total blood

Table 1. List of Aabian Oryx (*O. leucoryx*) blood samples from NWRC.

Sample number	Sex	Date of birth
1	Male	8/3/2003
2	Male	29/3/2003
3	Male	3/4/2003
4	Male	6/4/2003
5	Male	6/4/2003
6	Female	23/4/2003
7	Male	9/8/2004
8	Female	10/3/2003
9	Female	3/8/2004
10	Female	1/9/2004

(about 10 ml) was kept in ice for 30 min with 40 ml erythrocyte lysis buffer (155 mM NH4Cl, 10 mM KHCO3, 1 mM EDTA, pH 7.4). After this period, the samples were centrifuged at 1800 rpm for 15 min and the upper layer decanted. After the re-suspension of the "pellet" in 3 ml of lymphocyte lysis buffer (10 mM Tris–HCl, pH 8.0, 0.4 M NaCl, 2 mM EDTA, pH 8.2), 80 µg of K proteinase (10 mg/ml) and 300 µl of SDS 10% were added and carefully mixed. The samples were incubated overnight at 37°C. On the following day, 1 ml of saturated NaCl (6 M) was added and the samples were vigorously mixed for 15 s and centrifuged for 20 s at 2500 to 3000 rpm. The DNA was precipitated by adding two volumes of absolute ethanol (room temperature) and washed with 70% ethanol. After drying, the DNA was resuspended in TE10:1 (10 mM Tris–HCl, 1 mM EDTA, pH7.6).

DNA purity and quantity were determined by a spectrophotometer. Then, the DNA samples were diluted for final concentration of 20 ng/µl and stored at -20°C till use.

Primers used in RAPD analysis

A total of 23 decamer oligonucleotide primers obtained from *in vitro* gene-life technologies, universal primer sequences of Operon technologies, Gibco BRL ™ and University of British Colombia (UBC)) were used for the random amplified DNA polymorphism detection. The primers sequences are demonstrated in Table 2.

PCR reaction

2X PCR master mix from Fermentas®, Lithuania was used for the PCR reaction. Each reaction contained all the necessary reagents (dNTPs 200 nm of each and 0.6 unit of Taq DNA polymerase) except primers and DNA template for performing the 25 µl reaction. 50 ng of genomic DNA and 200 pmol of each random primer were added.

RAPD technique based on the polymerase chain reaction (PCR) was conducted to detect RAPD fragments. The thermal cycler was programmed by an initial strand separation cycle at 94°C for 5 min. The next 40 cycles composed of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and polymerization step at 72°C for 2 min. The final cycle was a polymerization cycle which was performed at 72°C for 7 min. Then, electrophoresis of samples was carried out using 1% agarose gels.

The PCR products of each reaction were analyzed by electrophoretic separation in 1.2% agarose gel. DNA marker of Gibco BRL (100 bp DNA ladder marker) was added on the sides of the gel to determine the DNA patterns. Gel was stained by ethidium bromide (0.5 mg/ml). A mixture of 25 µl reaction and 5 µl of loading buffer were loaded into the wells of the gel. Electrophoresis was run at 80 V for about 4 to 5 h. The resultant RAPD patterns were visualized using UV-transilluminator and photographed.

Table 2. RAPD primer sequences.

Number	Primer	Sequence 5'-3'
1	PR03	AGTCAGCCAC
2	PR04	AATCGGGCTG
3	PR05	AGGGGTCTTG
4	PR06	GGTCCCTGAC
5	PR07	GAAACGGGTG
6	PR11	CAATCGCCGT
7	PR12	TCGGCGATAG
8	PR13	CAGCACCCAC
9	PR14	TCTGTGCTGG
10	PR15	TTCCGAACCC
11	PR16	AGCCAGCGAA
12	PR17	GACCGCTTGT
13	PR20	GTTGCGATCC
14	PR23	TTGAGACAGC
15	PR28	TGGGGGACTC
16	PR30	CCGCATCTAC
17	PR31	GACGGATCAG
18	PR34	GACGCCACAC
19	PR35	ACCTTTGCGG
20	PR38	CAGCACCGCA
21	PR19	CAAACGTCGG
22	PR29	TTCCCCCGCT
23	PR33	CCAGTACTCC

DNA ladder size marker produced fragments of precisely known molecular weights namely: 1400, 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and100 bp. The mobility of each fragment, some of small fragments that could not be visualized, was measured and recorded and standard curve was drawn. Also, the mobility of each band produced from primer amplification of each sample was measured. The size in base pair of each fragment product was estimated using the standard curve. Microsoft excel 2007 (Microsoft Corporation, USA) computer program was used to draw the standard curve and to determine sizes.

Inter sequence simple repeats (ISSR) technique

Isolation of DNA

A modified method based on the protocol of Dellaporta et al. (1983) was conducted for the isolation of total genomic DNA from the blood samples. 10 ml blood placed on ice for 30 min with 40 ml erythrocyte lysis buffer (155 mM NH4Cl, 10 mM KHCO3, 1 mM EDTA, pH 7.4). After this period, the samples were centrifuged at 1800 rpm for 15 min and the upper layer decanted. The pellet was resuspended in 320 µl of the extraction buffer (10 Mm Tris-Hcl pH 8.0, 10 mM EDTA, 350 Mm Nacl, 0.5% SDS). The eppendorf tubes were incubated at 50°C for 20 min. Following, the sample was extracted by an equal volume of a standard phenol/chloroform/iso amyl alcohol, vortexed and spun for 2 min. The aqueous phase was

Table 3. DNA sequence of ISSR primers.

Number	Primer	Sequence 5'→3'
1	$(GAG)_3GC$	5'-GAGGAGGAGCG-3'
2	$(ACC)_6G$	5'-ACCACCACCACCACCACCG-3'
3	$(CACA)_5C$	5'- CACACACACACACACACAC -3'
4	$GTC(ACC)_6C$	5'-GTCACCACCACCACCCCACCC -3'
5	$(AG)_9C$	5'-AGAGAGAGAGAGAGAGAGC-3'
6	$(AGC)_6G$	5'-AGCAGCAGCAGCAGCAGCG-3'
7	$(AC)_9G$	5'- AGAGAGAGAGAGAGAGAGCG-3'
8	$(AC)_8CG$	5'-ACACACACACACACACCG-3'
9	$(AC)_8CCT$	5'-ACACACACACACACACCCT-3'
10	$(CAG)_5$	5'-CAGCAGCAGCAGCAG-3'
11	$(GTG)_5$	5'-GTGGTGGTGGTGGTG-3'
12	$(CA)_6AGG$	5'-CACACACACACAAGG -3'

transferred to a fresh 1.5 ml tube and then, was centrifuged in a microfuge at maximum speed for 10 min. Then, the aqueous phase was transferred to a fresh tube and 120 µl of 3 M sodium acetate and absolute ethanol were added. For precipitation, microfugation was carried out at maximum speed for 15 min, then, the pellet was washed with 70% ethanol and was dried in a speed vacuum drier. The dried pellet was dissolved in TE buffer depending on the size of the pellet. The concentration of the DNA was measured using UV-spectrphotometer and suitable dilution was done. Also, the genomic DNA was electrophoresed in agarose gel to evaluate its quality for further experiments. Finally, genomic DNA from each sample was adjusted to the concentration of 100 ng/ul for PCR analysis.

Primers used in ISSR analysis

For ISSR analysis, 12 primers were used (Table 3).

PCR reaction

Pre-made PCR reaction master mix green master mix (Promega) was used. The final total volume of each reaction was 25 µl; contained 0.625 units of Taq DNA polymerase, 2 mM $MgCl_2$ and 0.2 mM of each dNTPs. 50 ng of DNA and 1000 p.mol of the primer were added to the reaction

The thermocycler was programmed by an initial standard denaturation cycle at 94°C for 2 min. The following 35 cycles were composed of: denaturation step at 94°C for 1 min, annealing step was programmed at different temperatures according to the primer used (40 to 58 °C) for 30 s and elongation step at 72°C for 2 min. The final cycle was polymerization cycle performed at 72°C for 3 min.

The PCR products of each reaction were analyzed by electrophoretic separation in 1.5% agarose gel. DNA marker of Gibco (100 bp DNA ladder marker) was added on one side of the gel to determine the DNA patterns. Gel was stained by ethidium bromide (0.5 mg/ml). A mixture of 50 ml reaction and 10 ml loading buffer were loaded into the wells of the gel. Electrophoresis was run at 40 V for 5 h.

After electrophoresis, the ISSR patterns of the PCR products were visualized under UV light using UV-transilluminator and photographed. Sizes of ISSR fragments were determined by generating standard curve of DNA marker for each gel using 100 bp ladder. DNA ladder size marker produced fragments of precisely known molecular weights namely: 1500, 1400, 1300, 1200, 1100, 900, 800, 700, 500, 400, 300, 200 and 100. The mobility of each fragment was measured and recorded and the standard curve was drawn. Also, the mobility of each band produced from primer amplification of each sample was measured. The size in base pair of each fragment product was estimated using the standard curve. Microsoft Excel 2007 (Microsoft Corporation, USA) computer program was used to draw the standard curves and to estimate sizes.

RESULTS AND DISCUSSION

In this investigation, a fundamental question concerning the genetic variations of a representative sample of the Arabian Oryx from NWRC was addressed: Can genetic variation information be used to gain insight into Oryx fitness, which is an important step towards an exploitation of captive breeding program? To answer this question, many important experiments were carried out. These experiments included PCR-based RAPD and ISSR experiments. The results of the RAPD and ISSR primers used in this study confirmed that, there was no sex specificity for any of them. The characteristic bands were present in all the reaction products of the male and female samples. Therefore, the unambiguous polymorphic bands were scored in both sexes for their presence or absence in each RAPD as well as in the ISSR primer.

RAPD experiments

RAPD experiments were conducted using 25 random primers. Primers used in this study fell into three categories as follows; (1) 12 primers gave between 1 and 5 fragments namely; primers PR05, PR07, PR11, PR12, PR14, PR15, PR16, PR17, PR20, PR23, PR30 and PR31 in the ten Arabian Oryx genotypes; (2) eight primers gave 6 to 16 amplification products within the 10 genotypes namely, primer PR03, PR04, PR06, PR13, PR28, PR34, PR35 and PR38; (3) three primer gave no

Table 4. The primers used in the RAPD analysis of 10 Arabian Oryx genotypes, total number of fragments detected by each primer and number of polymorphic fragments.

Primer	Total scorable bands	Total number of fragment	Number of polymorphic fragment	Polymorphic fragment (%)	Fragment range size (bp)
PR03	46	6	5	83.3	1500-1850
PR04	48	13	13	100	500-1600
PR05	12	5	5	100	420-1200
PR06	46	11	11	100	90-700
PR07	5	4	4	100	400-1000
PR11	31	5	3	60	300-700
PR12	1	1	1	100	800
PR13	49	6	4	66.7	180-500
PR14	16	4	4	100	480-600
PR15	2	2	2	100	600-800
PR16	11	3	3	100	500-1000
PR17	5	1	1	100	780
PR20	15	4	4	100	1900-2200
PR23	4	4	4	100	900-1200
PR28	75	16	15	93.75	350-1450
PR30	12	4	4	100	180-600
PR31	30	5	4	80	380-800
PR34	46	7	6	85.78	500-900
PR35	53	9	9	100	500-1300
PR38	45	8	8	100	550-1200
Total	552	118	110		
Mean	27.6	5.9	10.5	93.5	90-2200

amplification product with all genotypes namely, primer PR19, PR29 and PR33.

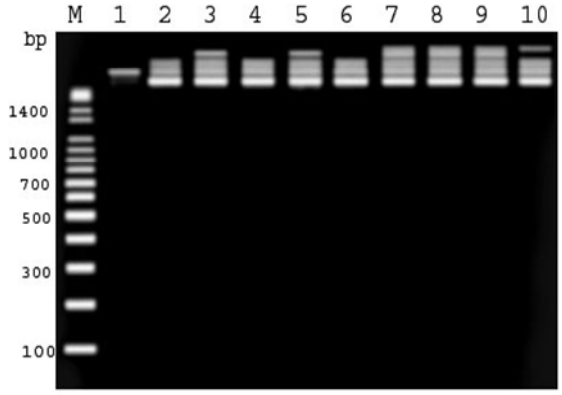
Table 4 shows that, 14 primers gave high level of polymorphism (100%). This means 70% of the primers studied showed 100% polymorphism. On the other hand, five primers gave lower level of polymorphism (30%). The average of polymorphism for all the primers was 93.5%. The total number of scorable fragments was 552 and the average was 27.6. Also, the average number of total fragments per primer was 5.9. The highest number of scorable fragments was given by PR28 (16 fragments) whereas, primers PR12 and PR23 gave the lowest number (1 to 4 fragments). The mean of polymorphic fragment was 10.5. The smallest fragment size detected was 90 bp with primer PR06, however, the largest fragment size detected was primer PR20 (2200 bp). Therefore, the sizes of the detected fragments by all the primers studied in the 10 Arabian Oryx genotypes were between 90 to 2200 bp.

This result indicated that, the number of detected bands was high enough to show that the experiments were conducted in a good manner. Also, the results were dependable and can be used for good conclusion. Also, the primers which showed high number of fragments (more than five) were more dependable than the rest of the fragments. The result showed that the best primer that can be used for further analysis is primer PR28, since it detected the largest number of fragments. At the same time, the average sizes of the fragments were normal in these types of RAPD experiments. Thus, we can conclude that this group of primers can be used as a set of markers for studying the captive herd of NWRC.

ISSR experiments

In this part of the research, the same 10 Arabian Oryx genotypes were used representing the current captive population of Saudi Arabian Oryx at NWRC. Currently, these herds are used as selecting material. This polymorphism study in this report was conducted using 12 ISSR primers. Among the molecular markers the ISSR analysis was chosen because of many advantages such as; it targets simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome and evolve rapidly and it includes amplification of specific and repeatable fragments (Sheppard and Smith, 2000).

The 12 primers used generated multiple fragments in the group of the 10 animals (Table 2). The DNA fragment profiles of the 12 primers are represented in Figure 2. Primer IS08 showed the highest number of polymorphic fragments (13 fragments) (Figure 2d) and primer IS02 showed the lowest number of polymorphic fragments (Figure 2c). The total number of detected fragments was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700
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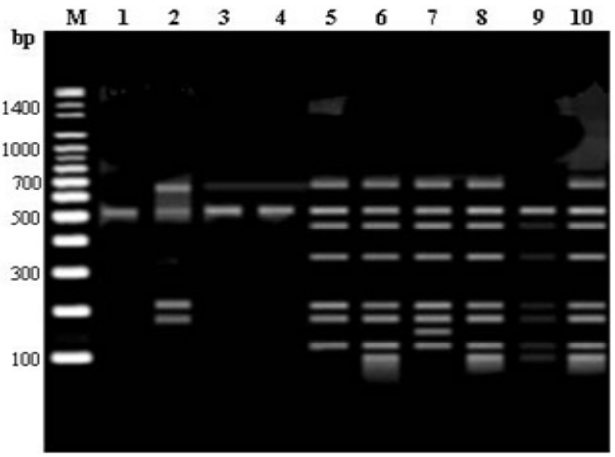
100

A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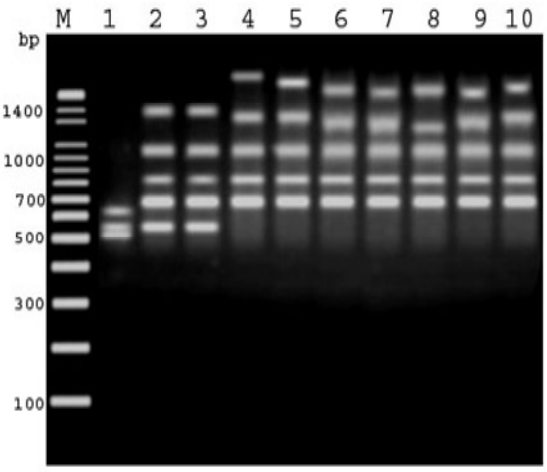
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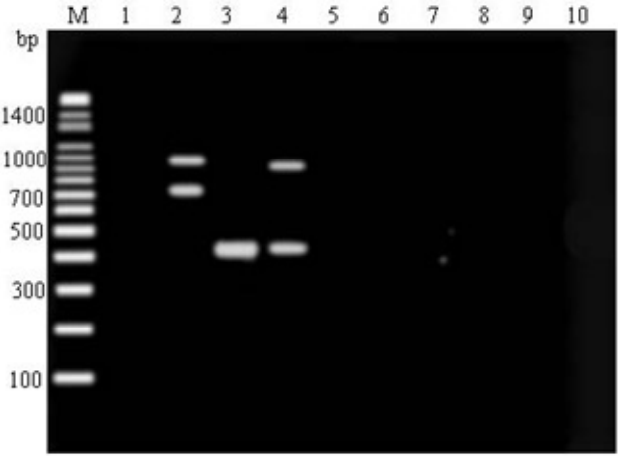
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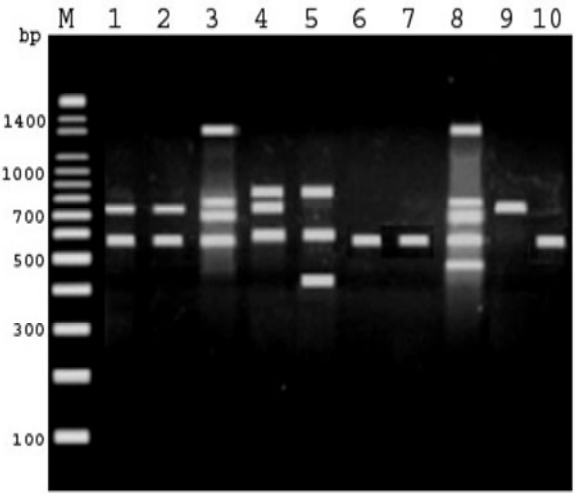
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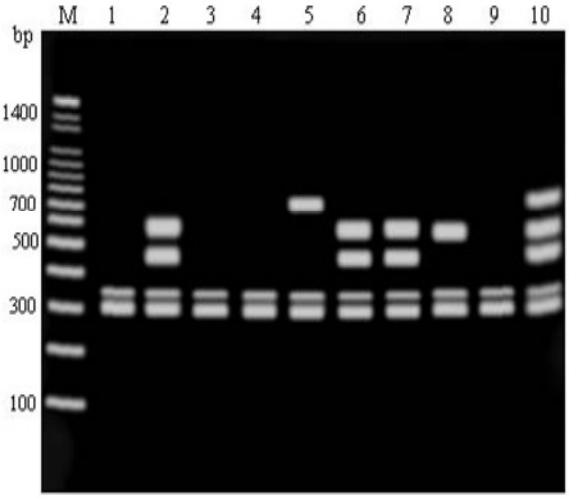
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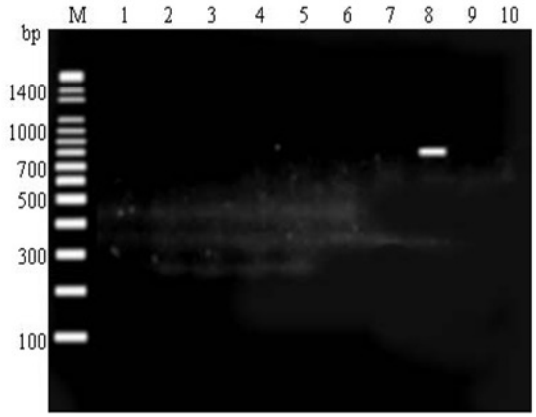
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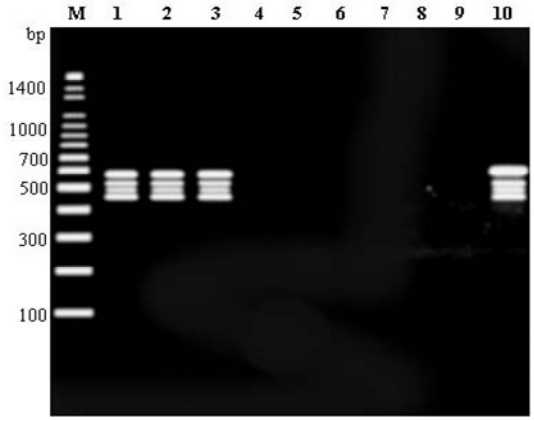
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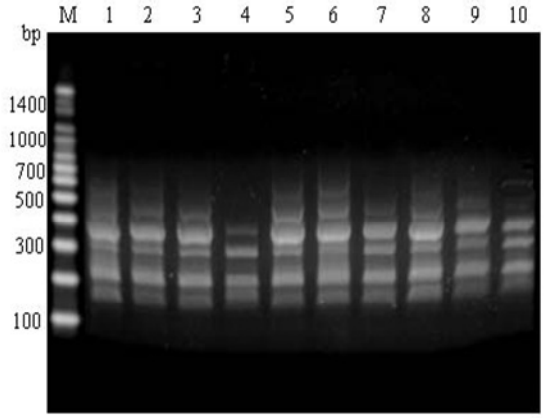
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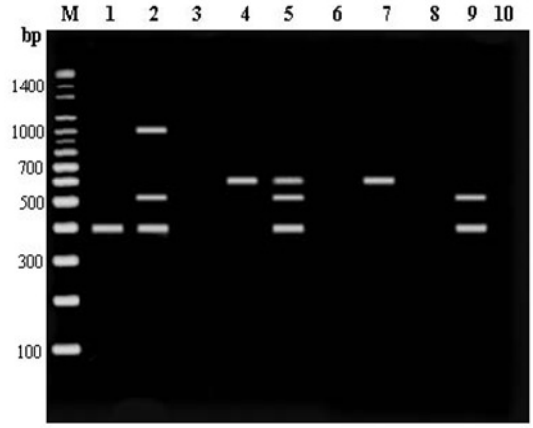
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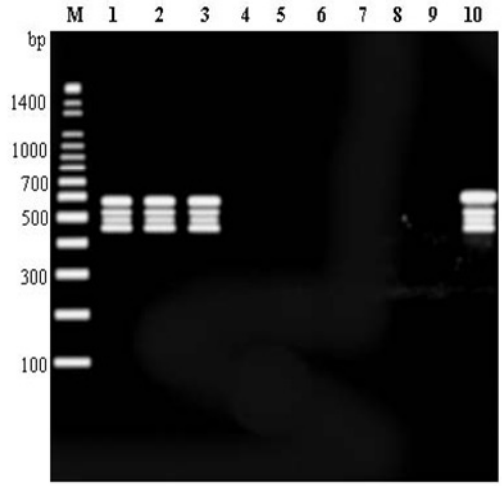
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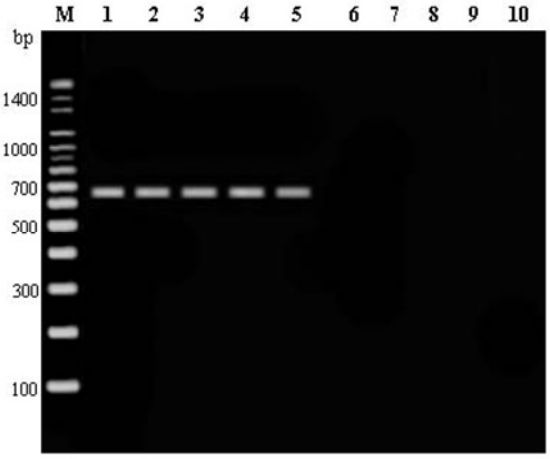
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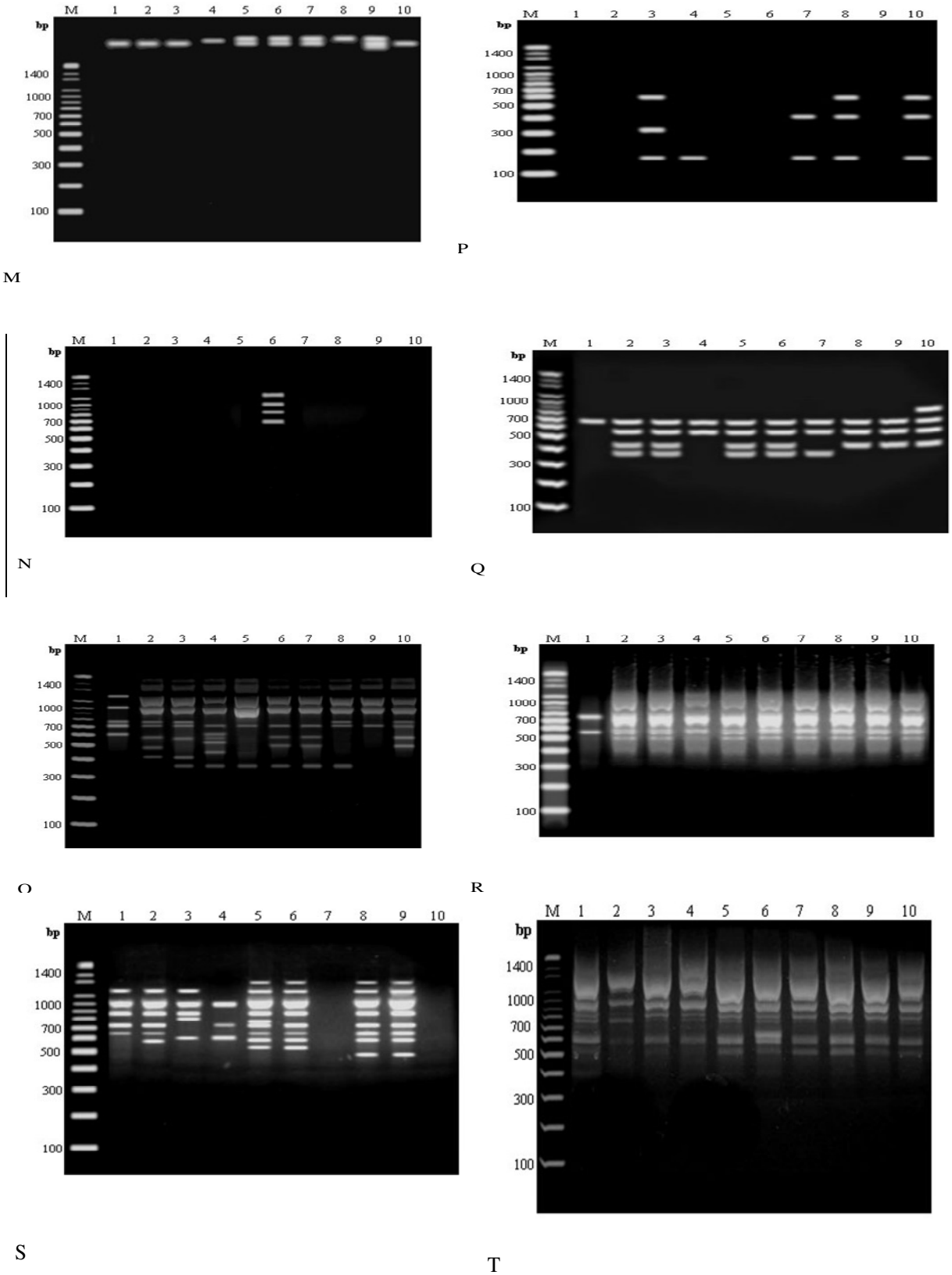
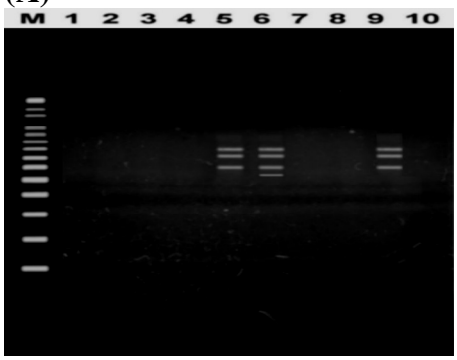
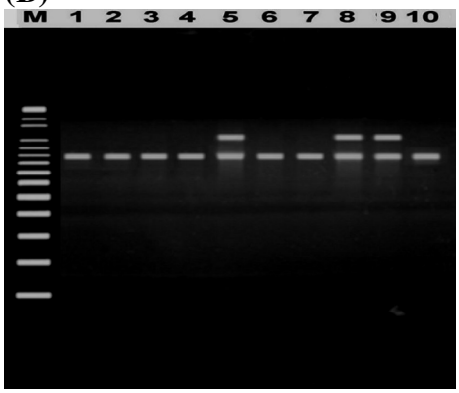


Figure 1. DNA fragments detected by RAPD primers: A, PR03; B, PR04; C, PR05; D, PR06; E, PR07; F, PR11; G, PR12; H, PR13; I, PR14; (J) PR15; (K) PR16; (L) PR17; (M) PR20; (N) PR23; (O) PR28; (P) PR30; (Q) PR31; (R) PR34; (S) PR35; (T) PR38. M is a DNA ladder.

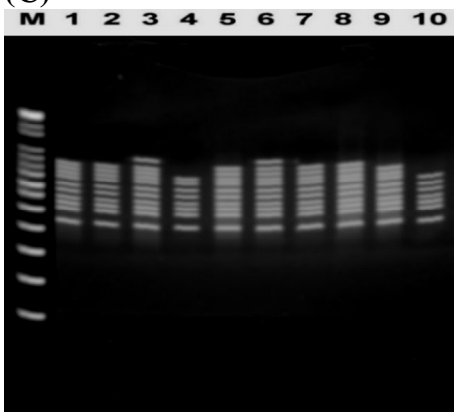
(A)


M 1 2 3 4 5 6 7 8 9 10

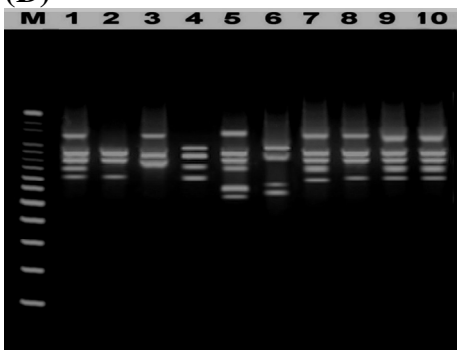
(B)


M 1 2 3 4 5 6 7 8 9 10

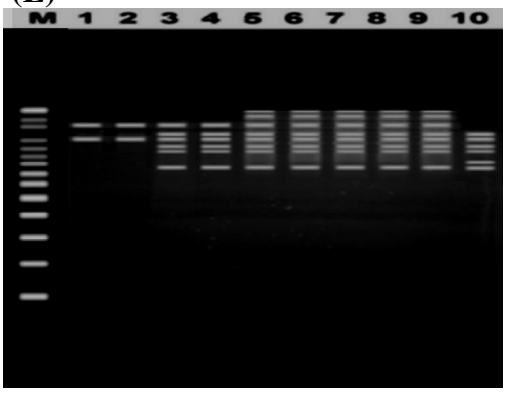
(C)


M 1 2 3 4 5 6 7 8 9 10

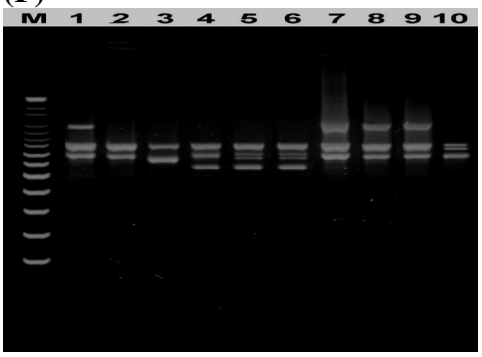
(D)


M 1 2 3 4 5 6 7 8 9 10

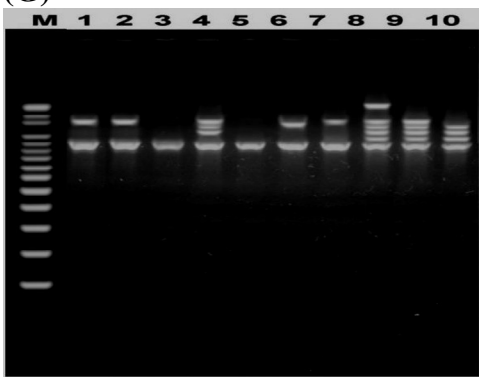
(E)


M 1 2 3 4 5 6 7 8 9 10

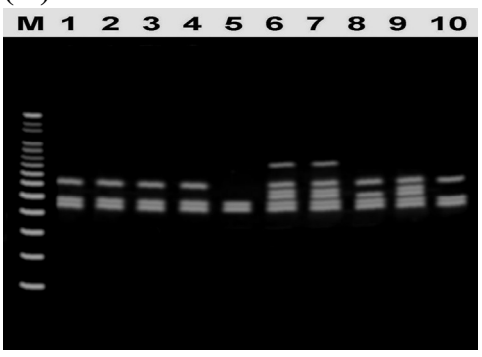
(F)


M 1 2 3 4 5 6 7 8 9 10

(G)


M 1 2 3 4 5 6 7 8 9 10

(H)


M 1 2 3 4 5 6 7 8 9 10

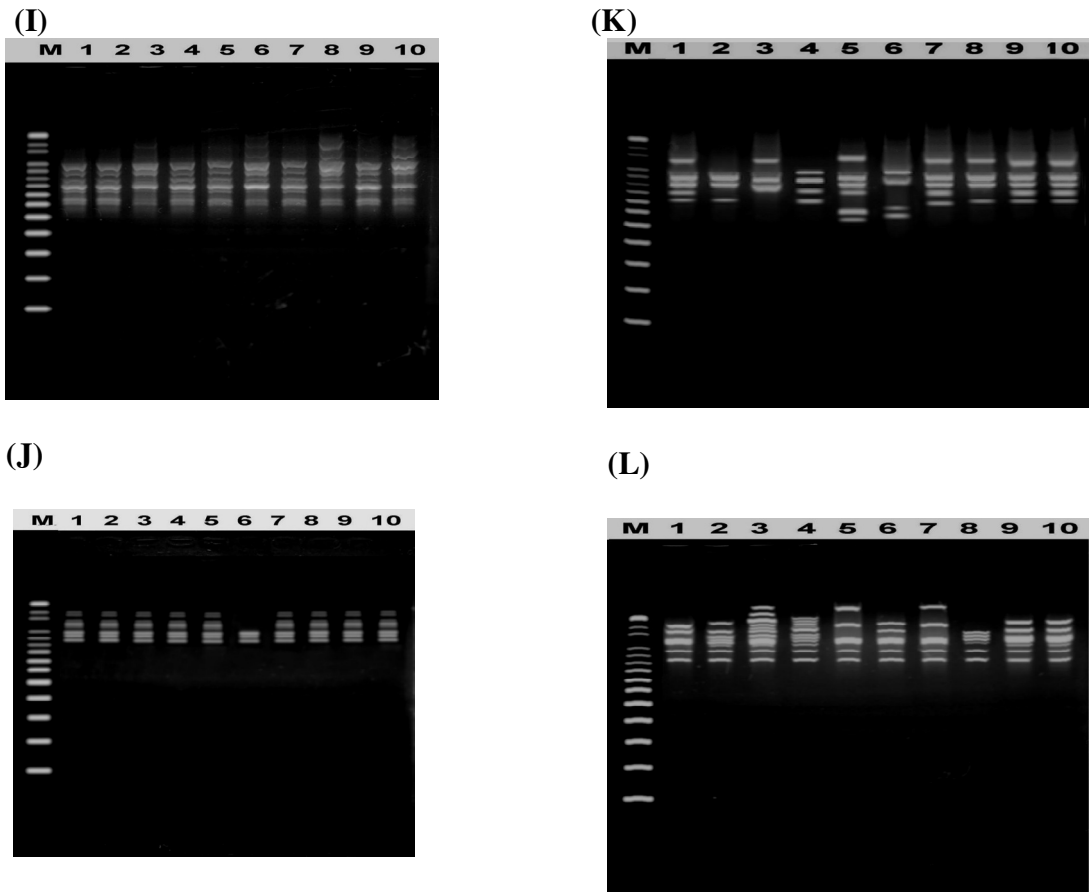


Figure 2. continues. DNA fragments detected by ISSR primers: (A) IS01; (B) IS02; (C) IS07; (D) IS08; (E) IS10; (F) IS11; (G) IS13; (H) IS15; (I) IS16; (J) IS17; (K) IS18; (L) IS19. M is a DNA ladder.

89 with an average of 8.09 fragments per primer and the range was between 2 to 13 fragments per primer (Table 5). The total number of detected fragment per primer was expected because it depended on the number of ISSR loci in each animal while, the total number of polymorphic fragment was 65 with an average of 5.42 per primer and the range was from 1 to 13 per primer. The percent of polymorphic fragments ranged from 27.27 to 100 with an average of 71.52 per primer. This data showed that, most of the detected loci were polymorphic which reflected the fact that the 10 animals studied came from wide diverse resources. The range of the fragment sizes was between 400 and 1600 bp which were relatively wide. This may be due to the type of primers used in this study. Thereafter, variability for each locus was measured. Each polymorphic fragment was scored as a locus with two allelic classes; therefore, the maximum heterozygosity value of an ISSR locus was 0.5. Finally, the average heterozygosity values for all detected loci for each primer were estimated. The average heterozygosity was 0.24 and the range was from 0. 09 to 0.36. This is an indication that the animals studied were relatively diverse.

The obtained result showed that, ISSR technique is a useful method for detecting polymorphism in Arabian Oryx. The simple inheritance of ISSR markers combined with the easiness of detecting polymorphic markers gave the system great potential in the studies of Arabian Oryx variabilities. Therefore, for the variability purpose studied, the 65 ISSR markers were tracked to be different among the animals. Many fragments were found to be unique for specific animals. As well as, by choosing the similar and the dissimilar fragments among all the genotypes, the detected 65 ISSR fragments indicated that, the primers tested were discriminated among all the animals. These results showed clearly that the ISSR primers used in this study were sufficient to fingerprint the 10 animals studied. These values indicated a wide range of genetic variability among the 10 animals and consequently, within the captive herd of NWRC. This result is in accordance with the result obtained by Vassart and Greth, (1991) using electrophoretic blood markers among the founder herd and with Marshall et al. (1999) who indicated high level of variations among the population of the herd. This wide range of genetic variability can be explained by the fact that, many foreign germplasms were introduced to the germplasm of the Arabian Oryx of NWRC. Also, this

Table 5: Levels of polymorphism detected by ISSR primers in 10 Arabian Oryx genotypes from NWRC.

No	Primer	Total number of fragments	Number of polymorphic fragments	Percent of polymorphic fragments	Range of fragment sizes (bp)	Heterozygosity
IS01	$(GAG)_3GC$	4	4	100	550-800	0.36
IS02	$(ACC)_6G$	2	1	50	900-1200	0.21
IS07	$(CACA)_5C$	11	3	27.27	450-1100	0.10
IS08	$GTC(ACC)_6C$	13	13	100	550-1300	0.31
IS10	$(AG)_9C$	9	8	88.99	750-1500	0.29
IS11	$(AGC)_6G$	6	5	83.33	650-1100	0.3
IS13	$(AC)_9G$	5	4	80	400-1400	0.32
IS15	$(AC)_8CG$	5	3	60	450-800	0.18
IS16	$(AC)_8CCT$	8	4	50	650-1400	0.19
IS17	$(CAG)_5$	6	3	50	950-1400	0.09
IS18	$(GTG)_5$	11	10	90.91	500-1300	0.25
IS19	$(CA)_6AGG$	9	7	77.78	980-1600	0.26
	Total	89	65			
	Mean	8.09	5.42	71.52	400-1600	0.24

range can be considered as a good potential source for the selection and genetic improvement which may lead to better conservation of Arabian Oryx.

Finally, this result of genetic variation of RAPD and ISSR revealed several features of differentiation among the 10 Arabian Oryx genotypes as a representative sample of NWRC captive herd. The first is the unique feature of the high level of variation among the 10 genotypes of NWRC. The second is, since, these samples are good representative of the NWRC captive breeding herd, it can be concluded that, the amount of variations among the NWRC germplasm is enough for the selection and breeding improvement without affecting the genetic diversity within the germplasms. The third is, although there were a large amount of variations, there is a chance that the breeder may encounter some animals which are similar, therefore, it is important for the breeder to measure the amount of variations among animals before starting any program of improvement and selection.

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