Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 59 (1): 125-133. Oct. 2018
DOI: 10.5455/ajvs.7699

Verifying parentage and genetic variability among Arabian horse using ISSR markers

Rania A. Tahseen[1, 2], Karam A. Amein[3], Dalia H. Samak[2], Yasser S. El-Sayed[2]*, Sherif M. Nasr[4]

[1] DNA Forensic and Diagnostic Laboratory, Faculty of Medicine, Assiut University, Assiut 71111, Egypt
[2] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt
[3] Department of Genetics, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt
[4] Department of Molecular Biology and Genetics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt

Key words:
Arabian horse; Paternity testing; DNA polymorphism; ISSR markers

***Correspondence to:**
Email: elsayed-ys@vetmed.dmu.edu.

ABSTRACT

The study aimed to verify parentage and genetic variability among twelve Arabian horses using five ISSR markers. Forty-five DNA loci, which extracted for the sire, mare and foal were amplified and replicated using the tested primers with an average of 9.0 loci per primer, and every marker is compared. The total number of polymorphic alleles was 33 (73.33% polymorphism), with an average of 6.6 alleles per primer. The markers (GAG)6G-ISSR (12 loci), (AGC)6C-ISSR (11 loci) and (AGC)6G-ISSR (9 loci) amplified the highest number of loci and possessed the highest PIC values indicating that they are the most promising for analysis of genetic variety and paternity in the Arabian horse. The highest similarity and shortest genetic distance (Si=0.926, Gd=0.077) were between Abeer and her daughter Karma. While, the lowest similarity and longest genetic distance (Si=0.353, Gd=1.041) between Sary and Farhan. The UPGMA cluster analysis supported the genetic relatedness as expected among the 12 Arabian horses. The dendrogram grouped the 12 Arabian horses according to their paternity, i.e. pedigree levels, into three main clusters within a branched-off 0.439 genetic similarity.

1. INTRODUCTION

About 3500 years ago, the Arabian horse became an essential component in the Egyptian empire. Using them to pull chariots allowed the Pharaohs rule to extend far beyond their own borders. The power and beauty of the mighty Egyptian Arabian have been both carved into hieroglyphs and written in the Bible. Breeding of these animals is taken very seriously, and bloodlines are carefully controlled to preserve the purity and desired characteristics. In the 19th, the ruling families of Egypt selected the finest horses from the deserts of Arabia and brought them to Egypt for breeding. Not since King Solomon has there been a collection that rivaled this one was the intention was to protect, preserve was to prolong this ancient bloodline while preserving their unique qualities. This collection was the foundation of the Egyptian Arabian, as we know them today (Wentworth, 1980, Wikipedia, 2018).

The need for paternity testing increases especially in natural breeders that had multiple estruses or when using artificial insemination where the original sir is not well known. In addition, it is not unusual to be unsure as to which horse is the biological father of a foal. Therefore, equine parentage testing is extremely important not only for horse breeds but also for owners to be able to guarantee the parentage of a foal. A real guarantee of pedigree can only be scientifically accurate if it the pedigree can be genetically proven. In the past, the pedigree was manually recorded, based on close observation. However, these traditional methods of recording ancestry and pedigree leave room for

many errors. Parentage DNA testing is highly accurate in cases where the dam, foal, and sire are analyzed (Glazko, 2008, Kurylenko and Suprun, 2015, Voronkova et al. , 2011).

With the emergence of the Polymerase Chain Reaction (PCR) and discovery of microsatellites, relatedness analysis, paternity testing and pedigree control, in general, have adopted the new technologies which have advantages over previously used methods such as blood typing (Binns et al. , 1995, Bowling et al. , 1997). The new generation of molecular – genetic markers, fragments of DNA, flanking by invert repeat of microsatellite loci (Inter-Simple Sequence Repeat, ISSR-PCR markers) allowed to estimate the polymorphism of multi-loci spectra, dispersed on different genome localities (Li et al. , 2002, Schlotterer, 2000). This technology is more easily adapted to the study of unique breeds where nucleotide sequences are unknown and less expensive than Simple Sequence Repeat (SSR)-PCR techniques used for microsatellite genotyping (Bardukov, 2010). ISSR analysis has also been applied for investigation of populations of aboriginal horse breeds. Di-nucleotide based ISSR markers used for analysis of horse populations in other research have demonstrated low efficiency because of a low number of amplified fragments (Kol and Lazebnyi, 2006). However, the use of trinucleotide primers for estimating genetic diversity of horse breeds has resulted in patterns with a large number of clear bands (Kuhl and Caskey, 1993, Voronkova et al., 2011). Microsatellite and ISSR markers were used to study the genetic diversity in Criollo and Marismeno horse population (Cortes et al. , 2017, Pablo Gómez et al. , 2016). In several studies including Arabian horses, ISSR markers have been extensively used for genetic relationship, fingerprint, and diversity (Ahmed et al. , 2018, Ivanovich et al. , 2012, Kurylenko and Suprun, 2015, Voronkova et al., 2011).

The present study aimed to verify parentage and genetic variability among Arabian horses (Equus caballus) using ISSR markers. The genetic distance, UPGMA clustering analysis, polymorphic information content (PIC) and Resolving power (Rp) were also calculated.

2.MATERIAL AND MESTODS

2.1. Arabian horses and samples collection:

Hair samples were collected from 12 Arabian horses, which raised in El Zahraa Stud, Egyptian Agricultural Organization (EAO), Ahmad Esmat Street, Ain Shams, Cairo, Egypt. The Arabian horses are namely; Barakat, Loly, Khemer, Karma, Abeer, Habiba, Farah, Sary, Malak, Amal, Abo-Trika, and Farhan. The pedigree of these horses was illustrated in Fig.1, based on breeding records provided by El Zahraa Stud. About 70 to100 hair samples were forcibly pulled from mane and tail hairs from each animal confirming that the hair follicles are attached as they are what contains the molecular material of horse.

2.2. DNA extraction

Using Genomic DNA kits isolate II, high-quality nuclear DNA was extracted in DNA Forensic and Diagnostic laboratory, Faculty of Medicine, Assiut University according to manufacturer′s instructions. DNA was spectrophotometrically quantified, then used for ISSR-PCR analysis based upon the standard protocol (Zietkiewicz et al. , 1994).

2.3. ISSR-PCR amplification

Five trinucleotide microsatellite core motifs with anchor nucleotides were used as primers (Table 1), based upon the standard protocol (Zietkiewicz et al., 1994). Polymerase chain reaction (PCR) was carried out using a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, U.K.) in the Department of Genetics, Faculty of Agriculture, Assiut University. The reaction conditions included initial denaturation (94°C, 4 min); followed by 32 cycles of denaturation (94°C, 30 s); annealing (58°C, 30 s); extension (72°C, 2 min); and final extension (72°C, 5 min). Amplification products were separated by electrophoresis conducted with a 1.5% agarose gel using 0.5× TBE-buffer at 100 V for 80 min. The DNA fragments were then stained with ethidium bromide and visualized on a Transilluminator (Ultra-Violet Product, Upland, CA, USA,). The DNA fragment sizes were determined using the molecular size marker 100bp DNA ladder H3 RTU (GeneDirsx).

2.4. Data analysis

The Multivariate Statistical Package (MVSP) software was used to visually score ISSR bands as present (1) or absent (0). Each DNA product of amplification considered as the separate DNA locus (Glazko, 2008). The genetic similarity (Si) and genetic distance (Gd) between the tested horses were calculated using the pairwise comparisons (Nei and Li, 1979). The dendrogram of genetic relationships based on similarity estimates was built by the MVSP software program using the unweighted pair-group method with arithmetic average (UPGMA).

In addition to the number of amplified bands (No. of loci), the percentage of polymorphic bands

(PPB), polymorphism information content (PIC) and resolving power (RP) were recorded for each ISSR primer. PIC indices were calculated as PIC = 2f(1 – f), where f is the frequency of one of the alleles. Since ISSR-PCR markers exhibit a dominant model of inheritance expressed as the presence of amplification product, f was calculated using the formula f = (R)½, where R, is the frequency of the horses lacking the DNA fragment of given size in their profile, among all horses. Then, the R-value is the proportion of homozygotes for recessive allele (Anderson et al. , 1993, Feofilov et al. , 2011). RP (indicates the discriminatory potential of each primer used) was estimated using the equation RP=ΣIb, where Ib is the informativeness of band and Ib= 1-[2 × (0.5 - p)], where p is the proportion of genotypes containing the band (Altıntaş et al. , 2008).

3.RESULTS

The five ISSR primers amplified a total of 45 ISSR-DNA fragments (loci) from the twelve Arabian horses with an average of 9.0 loci per primer (Table 2, Fig. 2). Only 12 (26.67%) of these bands were conserved among all Arabian horses which considered as species-specific DNA fragments, while 33 loci were polymorphic with the percentage of 73.33%. The highest percentage of polymorphism (PPB) was obtained by (AGC)6C-ISSR marker (90.91%) followed by (AGC)6G-ISSR (77.78%), (GAG)6G-ISSR (66.67%), (GTG)6A-ISSR (62.50%) and the lowest with the (ACC)6G-ISSR primer (60.00%). (GAG)6G-ISSR amplified a maximum of 12 loci followed by (AGC)6C-ISSR primer (11 loci), (AGC)6G-ISSR (9 loci), (GTG)6A-ISSR (8 loci) and (ACC)6G-ISSR primer (5 loci). The number of ISSR loci detected in the Arabian horses and amplified bands ranged from 20 in Loly and Habiba to 26 in Karma and Abeer (Table 2).

Combined analysis of ISSR data (Table 3) obtained by the five primers revealed that the highest similarity and shortest genetic distance (Si=0.926, Gd=0.077) between Abeer and Karma. Meanwhile, the lowest similarity and longest genetic distance (Si=0.353, Gd=1.041) between Sary and Farhan. The interrelationships between the twelve Arabian horses are illustrated in the UPGMA clustering analysis (Fig. 3) based on the combined data of the five ISSR primers. The dendrogram grouped the 12 Arabian horses according to their paternity, i.e. pedigree levels into three main clusters within a branched-off 0.439 genetic similarity. The first cluster was divided into two subclusters in which Barakat and his parents Abo-Trika and Habiba were grouped together in the 1st subcluster within Si=0.867 while Barakat was closer to his father Abo-Trika (Si=0.913) than his mother Habiba. In the 2nd subcluster, Abeer and her daughter Karma were clustered together firstly at Si=0.926 and then with their ancestor Khemer within Si=0.755.

Table (1): Description of the trinucleotide microsatellite core motifs with anchor nucleotides that were used as ISSR primer markers.

No.	Primer	Repeats	Nucleotide sequence (5'⟶3')
1	$(AGC)_6G$	$(AGC)_6$	AGC AGC AGC AGC AGC AGC G
2	$(GAG)_6G$	$(GAG)_6$	GAG GAG GAG GAG GAG GAG G
3	$(GTG)_6A$	$(GTG)_6$	GTG GTG GTG GTG GTG GTG A
4	$(AGC)_6C$	$(AGC)_6$	AGC AGC AGC AGC AGC AGC C
5	$(ACC)_6G$	$(ACC)_6$	ACC ACC ACC ACC ACC ACC G

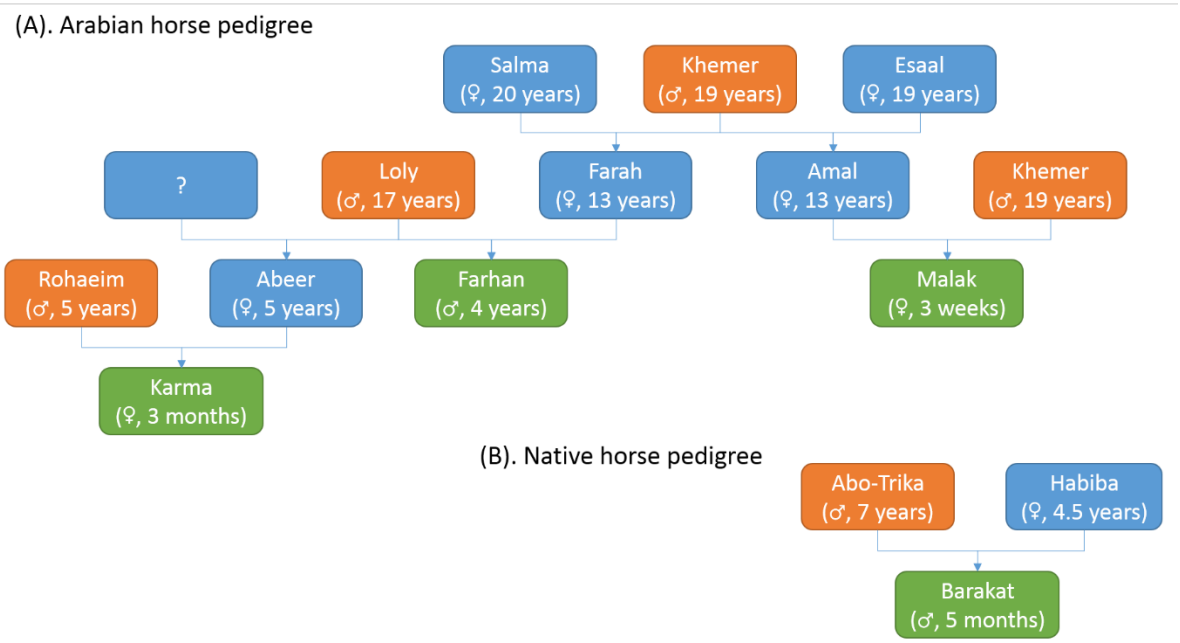

Figure 1: The pedigree of Arabian (A) and native (B) horses, in which Barakat, Loly, Khemer, Karma, Abeer, Habiba, Farah, Sary, Malak, Amal, Abo-Trika, and Farhan were used in the present study. Yellow: sir, Blue color: mare, Green color: foal.

Table (2): Number of amplified and polymorphic DNA-fragments (loci), the percentage of polymorphic bands (PPB), polymorphism information content (PIC) and resolving power (RP) generated from twelve Arabian horses by five ISSR primers.

Primer	Barakat	Loly	Khemer	Karma	Abeer	Habiba	Farah	Sary	Malak	Amal	Abo-Trika	Farhan	No of Amplified Bands (loci)	No. of polymorphic bands (loci)	PPB	PIC	RP
$(AGC)_6G$	5	4	6	5	6	4	5	4	4	3	4	4	9	7	77.78	0.33	5.00
$(GAG)_6G$	6	4	5	6	5	5	5	7	5	7	5	5	12	8	66.67	0.32	2.83
$(GTG)_6A$	6	4	6	6	6	5	6	6	4	6	6	4	8	5	62.50	0.24	4.83
$(AGC)_6C$	4	5	5	5	5	4	5	4	5	4	4	6	11	10	90.91	0.37	7.33
$(ACC)_6G$	2	3	2	4	4	2	3	3	3	3	2	3	5	3	60.00	0.29	1.67
Total	**23**	**20**	**24**	**26**	**26**	**20**	**24**	**24**	**21**	**23**	**21**	**22**	**45**	**33**	**73.33**	**0.31**	**4.33**

3.1. Polymorphism information content (PIC)

The PIC was widely used to estimate the usefulness of the molecular marker. The PIC values were 0.33, 0.32, 24, 0.37 and 0.29 for the (AGC)6G-ISSR, (GAG)6G-ISSR, (GTG)6A-ISSR, (AGC)6C-ISSR and (ACC)6G-ISSR primers with an average of 0.31, respectively (Table 3).

3.2. Resolving power (RP)

The RP provides data on the aptitude of a primer to reveal the genetic or taxonomic relationships of a group of genotypes under study. The highest RP value was perceived with the (AGC)6C-ISSR (7.33) followed by (AGC)6G-ISSR (5.00), (GTG)6A-ISSR (4.83), (GAG)6G-ISSR (2.83) and the lowest (ACC)6G-ISSR (1.67) with an average RP of 4.33 per primer (Table 3).

Table (3): Genetic similarity (below the diagonal) and distance (above the diagonal) values calculated from the DNA fragments amplified from twelve Arabian horses with five ISSR primers.

	Barakat	Loly	Khemer	Karma	Abeer	Habiba	Farah	Sary	Malak	Amal	Abo-Trika	Farhan
Barakat	--	0.425	0.476	0.457	0.457	0.139	0.567	0.858	0.870	0.726	0.091	0.594
Loly	0.654	--	0.462	0.351	0.351	0.302	0.368	0.981	0.768	0.837	0.345	0.288
Khemer	0.621	0.630	--	0.322	0.241	0.462	0.511	0.787	0.901	0.858	0.405	0.726
Karma	0.633	0.704	0.724	--	0.077	0.442	0.489	0.944	0.962	0.819	0.387	0.726
Abeer	0.633	0.704	0.786	0.926	--	0.442	0.405	0.944	0.962	0.819	0.387	0.511
Habiba	0.870	0.739	0.630	0.643	0.643	--	0.560	0.457	0.768	0.728	0.146	0.587
Farah	0.567	0.692	0.600	0.613	0.667	0.571	--	0.693	0.693	0.567	0.499	0.534
Sary	0.424	0.375	0.455	0.389	0.389	0.419	0.500	--	0.223	0.128	0.794	1.041
Malak	0.419	0.464	0.406	0.382	0.382	0.464	0.500	0.800	--	0.274	0.803	0.837
Amal	0.484	0.433	0.424	0.441	0.441	0.483	0.567	0.880	0.760	--	0.660	0.901
Abo-Trika	0.913	0.708	0.667	0.679	0.679	0.864	0.607	0.452	0.448	0.517	--	0.624
Farhan	0.552	0.750	0.484	0.655	0.600	0.556	0.586	0.353	0.433	0.406	0.536	--

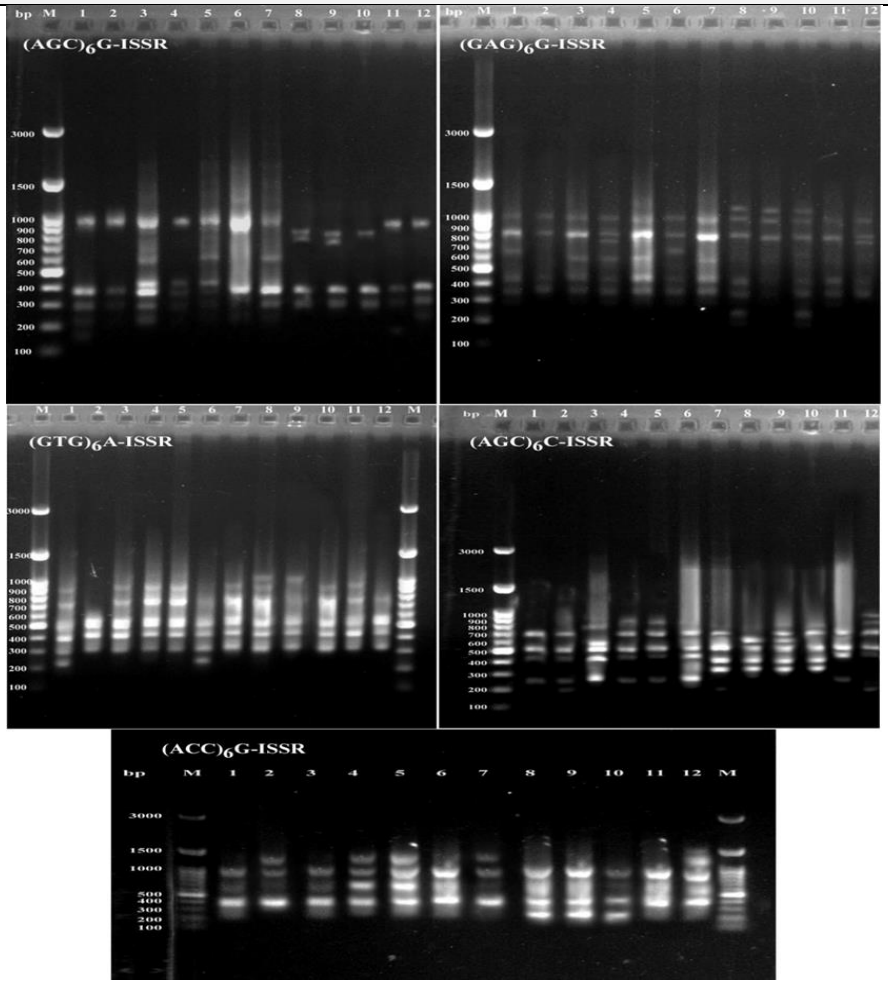

Figure 2: Agarose gel electrophoresis of ISSR profile in the 12 Arabian horses obtained by (AGC)6G-ISSR marker. M: DNA Ladder, (1): Barakat, (2): Loly, (3): Khemer, (4): Karma, (5): Abeer, (6): Habiba, (7): Farah, (8): Sary, (9): Malak, (10): Amal, (11): Abo-Trika, (12): Farhan.

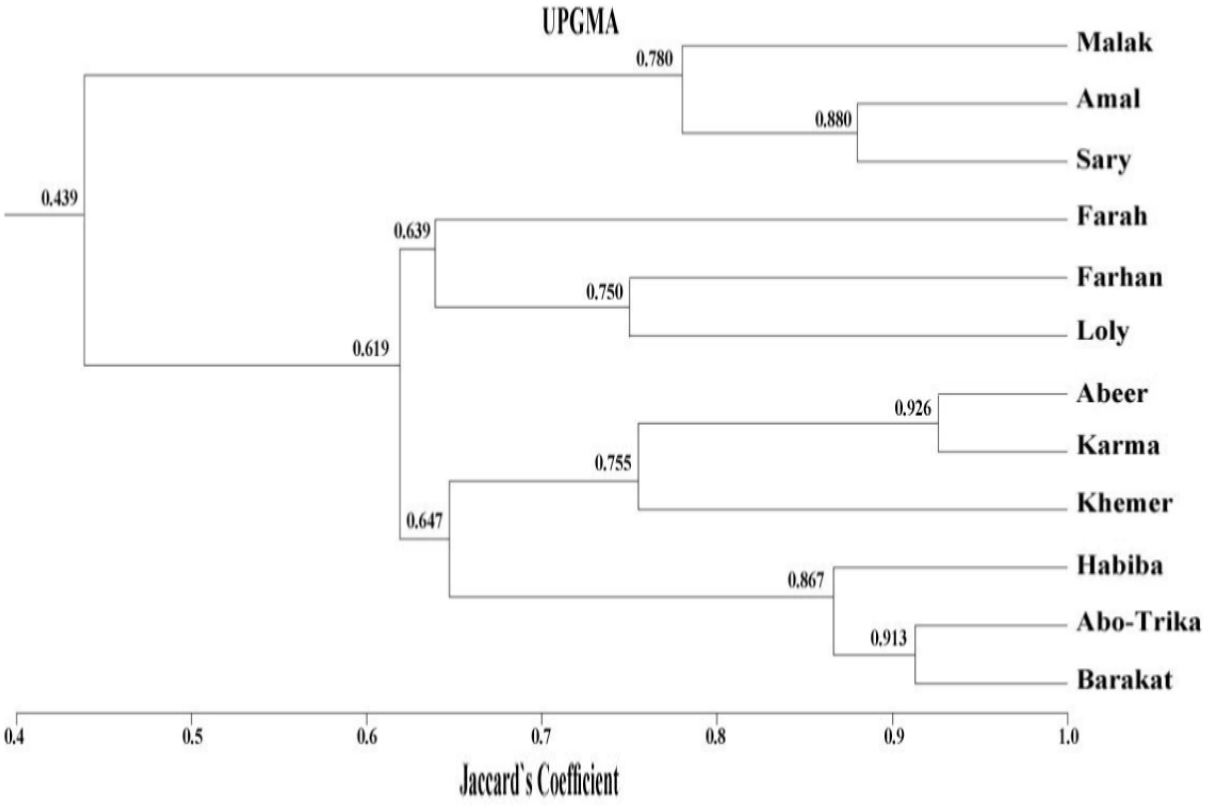

Figure 3: Dendrogram demonstrating the relationships among twelve Arabian horses based on data recorded from polymorphism of ISSR markers generated by five primers.

4. Discussion

Recent studies focused on genetic analysis of different horse breeds, especially the breeds with commercial value, in order to enhance their breeding efficiency and to maintain the genet diversity (Ahmed et al., 2018, Bigi and Perrotta, 2012, Cortes et al., 2017, Lindgren, 2001). Molecular markers are extensively used to identify breed differences, genetic and higher taxonomic variations among the population. The ISSR markers are single-stranded, complementary to the sequences of microsatellite and usually from 4 to12 repeat units. They amplify DNA sequences in-between the inverted repeats of the microsatellites and also known as ISSR fingerprinting. These markers are widely used in plant genetics and have informative in animal genetics and diversity studies (Velu et al. , 2008). The ISSR markers are also useful in conservation programs which enable to reduce the inbreeding and genetic diversity (Bigi and Perrotta, 2012). Furthermore, microsatellite and ISSR markers were used to study the genetic diversity in Criollo and Marismeno horse population (Cortes et al., 2017, Pablo Gómez et al., 2016). In several studies including Arabian horses, ISSR markers have been extensively used for genetic relationship, fingerprint, and diversity (Ahmed et al., 2018, Ivanovich et al., 2012, Kurylenko and Suprun, 2015, Voronkova et al., 2011).

Moreover, equine parentage testing is extremely essential for horse breeds and owners because it ensures correctness of breeding records, as well as verifying and guaranteeing lineages and guarding breed integrity. DNA Parentage among horses can be ultimately recognized using PCR technology to compare allele sizes. Herein, DNA isolated from 12 Arabian horses was subjected to ISSR analysis using five trinucleotide markers to study the paternity and genetic relatedness among them. The use of ISSR markers of trinucleotide repeats enabled generation of a profile with the great number of fragments, including polymorphic ones (Ahmed et al., 2018, Voronkova et al., 2011), confirming that the trinucleotide-based ISSR primers have been more informative than the dinucleotide ones.

A total of 45 DNA loci were amplified and replicated by the tested primers with an average of 9.0

loci per primer. Ahmed et al. (2018) used the informativeness of 12 multilocus ISSR markers to analyze the Arabian horse breeds including (28) Middle Eastern and (10) mixed (hybrid) Arabians. They found that the tested 12 ISSR makers amplified a total of 136 alleles and ranged from 9 to 18 alleles per primer. In this study, the amplified DNA fragments were detected between 165bp with (AGC)6G-ISSR to 1300bp with (ACC)6G-ISSR primer. The highest number of amplified DNA fragments attained with (GAG)6G-ISSR marker (12 loci) followed by (AGC)6C-ISSR marker (11 loci) denoting highly polymorphic ISSR markers (8 and 10 bands, respectively) and its usefulness in the genetic analysis of Arabian horses. Voronkova et al. (2011) compared the informativeness of six different types of ISSR markers to assess the inherited diversity of Mongolian, Buryat and Tuvinian horse breeds. They found that the trinucleotide markers amplified 20 (GAG-ISSR), 18 (ACC-ISSR) and 15 (CAC-ISSR) loci (fragments) from the tested horses. The total number of polymorphic alleles detected was 33 (73.33% polymorphism), with an average of 6.6 alleles per primer. These polymorphic loci are valuable in studying the genetic relatedness among the tested Arabian horses. They also found that the percentage of polymorphic loci obtained by trinucleotide ISSR markers were 95.0% (GAG-ISSR), 94.5% (ACC-ISSR) and 12.5% (CAC-ISSR) in the tested horse breeds.

The (ACC)6G-ISSR primer amplified the lowest number of DNA loci (5 loci) and lowest number of polymorphic loci (3 loci) reflecting that it is not ideal in the genetic analysis of horse breeds. For the (AGC)6C-ISSR primer, (GAG)6G-ISSR and (AGC)6G-ISSR markers, the high number of amplified fragments (11, 12 and 8, respectively), along with high number of polymorphic loci (10, 8 and 7, respectively) were obtained, making these markers most promising for analysis of genetic diversity and paternity in Arabian horse. Similar results were also obtained by Ahmed et al. (2018), Voronkova et al. (2011). In seven populations of Ukrainian horses including Arabian, Kurylenko and Suprun (2015) found that the 4 ISSR primers [(AGC)6G, (ACC)6G, (GTG)6A, (AGC) 6C] can be used for determination of a genetic distance between breeds and searching for phylogenetic connections. Use of these markers will also allow for an expansion of the pool of information about genetic variations among horse breeds. The genetic similarity and distance express the degree of divergence and relatedness between the tested horses. The highest similarity and shortest genetic distance (Si=0.926, Gd=0.077) between Abeer and her daughter Karma. While, the lowest similarity and longest genetic distance (Si=0.353, Gd=1.041) between Sary and Farhan.

The UPGMA cluster analysis supported the genetic relatedness as expected among the 12 Arabian horses. The dendrogram grouped the 12 Arabian horses according to their paternity, i.e. pedigree levels into three main clusters within a branched-off 0.439 genetic similarity. Barakat and his parents Abo-Trika and Habiba were separated together in one subcluster while Barakat was closer to his father Abo-Trika than his mother Habiba. In the 2nd subcluster, Karma and her mother Abeer were closely clustered together firstly and then with their ancestor Khemer. Similarly, Farhan and his father Loly followed by his mother Farah were separated together in the 2nd cluster, indicating that Farahan was highly similar to his father than his mother at the tested ISSR loci. Malak and her parents Sary and Amal were grouped together in a separate cluster where Amal and Sary were highly similar to each other than to their daughter Malak at the tested ISSR loci. These results of the present investigation showed that the tested ISSR markers were most informative for authentication of parentage, genetic distance and relatedness among Arabian horses. In addition, ISSR-PCR assay is rapid and reproducible generating adequate polymorphisms to provide the potential for large scale DNA typing applications. ISSR markers have been used to evaluate the genetic relationship and diversity in among horses (Ahmed et al., 2018, Cortes et al., 2017, Kurylenko and Suprun, 2015, Pablo Gómez et al., 2016, Voronkova et al., 2011). Moreover, it has been found that the combinations of certain DNA fragments in ISSR profiles successfully distinguished genomes of the Altaic from the trotting horse breeds (Feofilov et al., 2011).

Polymorphism information content (PIC): The ISSR markers revealed rational polymorphic loci and PIC value, which served as a polymorphism unit as well as a gene mapping tool for genetic diversity assays (Mehta et al. , 2007). Therefore, they are appropriate for assessing polymorphism analyses and to discriminate closely related and isolated animal species (Stolpovsky et al. , 2014). In addition, the PIC value is an indicative of the usefulness of the molecular marker in the analysis of genetic diversity and paternity. The PIC values obtained here are typical of dominant markers i.e. RAPD, AFLP®, which are comparatively lower than co-dominant marker systems i.e. SSR and RFLP (Vuylsteke et al. , 1999). In the present study, the PIC

values were 0.33, 0.32, 24, 0.37 and 0.29 for the (AGC)6G-ISSR, (GAG)6G-ISSR, (GTG)6A-ISSR, (AGC)6C-ISSR and (ACC)6G-ISSR primers with an average of 0.31, respectively. These results showed that (AGC)6C-ISSR, (AGC)6G-ISSR and (GAG)6G-ISSR markers possessed the highest PIC values and were the most informative for evaluation of genetic variability among the Arabian horses. Our PIC values were similar with one of microsatellites studies, where PIC ranging from 0.07- 0.3 (Criscione et al. , 2015). Ahmed et al. (2018) also found that the PIC values were ranged from 0.07 to 0.48 with an average of 0.2994.

Resolving power (RP): The resolving power (RP) was used to measure the ability of primers or techniques to distinguish between genotypes and to reflect the taxonomic relationships among them (Prevost and Wilkinson, 1999). The RP index provides a moderately accurate estimate of the number of genotypes identified by a primer. In the present study, the highest RP value was observed with the (AGC)6C-ISSR indicating that such primer is most promising for analysis of genetic diversity and paternity in the Arabian horse. In contrast, the (ACC)6G-ISSR primer displayed the lowest RP value reflecting that it is not optimal in the genetic analysis of horse breeds.

5. REFERENCES

Ahmed MMM, Sheikh A, Mutwakil MHZ, Saini KS, Alsulaimany FAS, EL Hanafy AA, et al. Comparative molecular analysis of issr markers in arabian horse breeds. J Anim Plant Sci. 2018;28:332-336.

Altıntaş S, Toklu F, Kafkas S, Kilian B, Brandolini A, Ö zkan H. Estimating Genetic Diversity in Durum and Bread Wheat Cultivars from Turkey using AFLP and SAMPL Markers. Plant Breeding. 2008;127:9—14.

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. Optimizing parental selection for genetic linkage maps. Genome. 1993;36:181-186.

Bardukov NV. ISSR-PCR profiles in trotter horses Izv. TSHA. 2010;6:152-157.

Bigi D, Perrotta G. Genetic structure and differentiation of the Italian catria horse. J Hered. 2012;103:134-9.

Binns MM, Holmes NG, Holliman A, Scott AM. The identification of polymorphic microsatellite loci in the horse and their use in thoroughbred parentage testing. Br Vet J. 1995;151:9-15.

Bowling AT, Eggleston-Stott ML, Byrns G, Clark RS, Dileanis S, Wictum E. 1997. Validation of microsatellite markers for routine horse parentage testing. Anim. Genet. 28:247-252.

Cortes O, Dunner S, Gama LT, Martinez AM, Delgado JV, Ginja C, et al. The legacy of Columbus in American horse populations assessed by microsatellite markers. J Anim Breed Genet. 2017;134:340-350.

Criscione A, Moltisanti V, Chies L, Marletta D, Bordonaro S. A genetic analysis of the Italian Salernitano horse. Animal. 2015;9:1610-1616.

Feofilov AV, Bardukov NV, Glazko VI. Gene pool differentiation between Altaic and trotting horse breeds inferred from ISSR-PCR marker data. Genetika. 2011;47:1230-1235.

Glazko VI. Divergence of domestic and wild species on different genome elements. J Agrobiol. 2008;25:41-3.

Ivanovich GV, Vladimirovich BN, Vladimirovich PA, Petrovich ST, Aleksandrovna EM, Theodorovna GT. Polymorphism of ISSR and IRAP markers in genomes of Musk-Oxen (Ovibos moschatus) and horse (Equus caballus) of Altaic breed. IZVESTIYA TSKhA. 2012:16-26.

Kol NV, Lazebnyi OE. Polymorphism of ISSR-PCR markers in Tuvinian population of reindeer Rangifer tarandus L. Genetika. 2006;42:1731-4.

Kuhl DPA, Caskey CT. Trinucleotide repeats and genome variation. Curr Opin Genet Dev. 1993;3:404-7.

Kurylenko U, Suprun I. Selection of optimal ISSR-marker systems for horses in the Ukraine. Agrarni Nauki. 2015;7:111-6.Li YC, Korol AB, Fahima T, Beiles A, Nevo E. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol Ecol. 2002;11:2453-65.

Lindgren G. Genome analysis in the horse. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology, Uppsala, Sweden: Uppsala University; 2001.

Mehta S, Goyal A, Sahani MS. Microsatellite markers for genetic characterisation of Kachchhi camel. IJBT. 2007;6:336-9.

Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America. 1979;76:5269-5273.

Pablo Gómez M, Landi V, Martínez AM, Gómez Carpio M, Nogales Baena S, Delgado Bermejo JV, et al. Genetic diversity of the semi-feral Marismeño horse breed assessed with microsatellites. Ital J Anim Sci. 2016;16:14-21.

Prevost A, Wilkinson MJ. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. TAG Theoretical and Applied Genetics. 1999;98:107-12.

Schlotterer C. Evolutionary dynamics of microsatellite DNA. Chromosoma. 2000;109:365-371.

Stolpovsky YA, Kol NV, Evsyukov AN, Nesteruk LV, Dorzhu Ch M, Tsendsuren T, et al. Comparative analysis of ISSR markers polymorphism in populations of yak (Bos mutus) and in F1 hybrids between yak and cattle in the Sayan-Altai region. Genetika. 2014;50:1163-1176.

Velu D, Ponnuvel KM, Muthulakshmi M, Sinha RK, Qadri SM. Analysis of genetic relationship in mutant silkworm

strains of Bombyx mori using inter simple sequence repeat (ISSR) markers. J Genet Genomics. 2008;35:291-297.

Voronkova VN, Tsedev T, Sylimova GE. Comparative analysis of the informativeness of ISSR markers for estimating genetic diversity of horse breeds. Genetika. 2011;47:1131-1134.

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, et al. Two high-density AFLP ® linkage maps of Zea mays L.: analysis of distribution of AFLP markers. TAG Theoretical and Applied Genetics. 1999;99:921-35.

Wentworth L. The Authentic Arabian Horse. 3rd Revised ed. Canaan, New York: HarperCollins Publishers Ltd; 1980.

Wikipedia. Arabian horse - Wikipedia. 2018.

Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20:176-183.