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DNA Fingerprinting of Iranian Arab Horse Using Fourteen Microsatellites Marker

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Abstract: Analysis of microsatellite markers as useful polymorphic genetic variations helps to characterize different animal species and breeds, moreover with develop molecular genetics techniques, beings identification and parentage testing is possible using a lot of molecular markers. So in the present study, the researchers examine the 14 genetic markers AHT5, AHT4, ASB23, ASB17, ASB2, VHL20, CA425, HMS7, HMS6, HMS3, HMS1, HTG4, HTG10 and LEX3 of Iranian Arab horses and use they in identity testing it species in Iran. To achieve this goal, DNA was extracted from blood samples collected from 13 families of Iranian Arab strain and then the multiplex polymerase chain reaction was used for amplification of fourteen markers with the specific primers and the PCR products were resolved on a non-denaturing 10% polyacrylamide gel by electrophoresis. The PCR products also remaining obtainable with formamide and electrophoresis was carried out on an ABI PRISM 3100 genetic analyzer using the recommended protocols. The average of heterozygocity was 0.656 and the expected of heterozygocity at this population was 0.697. Consequently, it seems that these fourteen markers can be used as an applicable marker for identifying Arabian horse.

Key words: Iranian Arab breed, microsatellites, STR, horse, primers, Iran

INTRODUCTION

The Arab breeder is one of the most influential horse breeds in the world. It is distributed worldwide and has been involved in the formation of many other horse breeds, such as the Thoroughbred (Bowling and Ruvinsky, 2000). In animal, breeding accurate determination of relatedness and efficient control of pedigree registration is of great importance. The identification of pedigree information is one of the difficulties in implementing at breeding programs in horse (Luís *et al*., 2002; Lee and Cho, 2006).

Recently, breeders have turned to molecular biology and use PCR (Polymerase Chain Reaction) for detection of short sequence repeats which are also referred to as microsatellites. Microsatellites (SSR or STR) are highly polymorphic genetic markers with co-dominantly inherited alleles that are relatively easy to score. Microsatellites are repeat regions of 2-7 nucleotide units that occur primarily in non-coding regions of DNA (Luis *et al*., 2002; Rhyu, 1996). Microsatellites have been used for linkage map construction, population genetics, molecular evolution studies, forensic sciences and as parentage testing markers (Tozaki *et al*., 2003).

The designation and number of microsatellites that should be used in parentage testing is yet a matter of discussion and depends on the characteristics of each locus and on the variability of the breed under study (Jakabova *et al*., 2002). STRs are simple sequences of DNA consisting of short tandem repeats have a high polymorphism and for this reason, they are used as powerful tools for recognizing the identity. These sequences the total 20% of constitute in Mammalia (Shiue *et al*., 1999; Cervantes *et al*., 2008). The objective of the present study was to perform a routine DNA typing with fourteen microsatellite markers for parentage verification and individual identification of Iranian Arabian horse.

MATERIALS AND METHODS

Sampling and DNA purification: Blood samples collected from 13 families of Iranian Arab strain in Southwest part of Iran. Blood collected in tubes coated with Na_2-EDTA and transferred to lab for DNA extraction. DNA extraction has conducted with use of DNA-kit (Genomic DNA Purification kit, Sinagen, Iran). Out of thirteen families, only two cases including sire, dam and foal selected for parentage verification test using genetic markers.

Analysis of DNA: The multi-plex Polymerase Chain Reaction (PCR) was used for amplification of 14 markers introduced by International Society for Animal Genetics (ISAG). The primer sequences used for the amplification of the loci are shown in Table 1. PCR was performed in a total volume of 25 μL of the following mixture: 20 ng of

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Table 1: Primer sequences used for amplification of the microsatellites marker

Locus	Primer sequences (5'-3')	Allele	Product size	References
AHT4	(F) 5′-AACCGCCTGAGCAAGGAAGT -3′ (R) 5′-GCTCCCAGAGAGTTTACCCT -3′	H, J, K, L, O	170-138	Binns *et al.* (1995)
AHT5	(F) 5′-ACGGACACATCCCTGCCTGC -3′ (R) 5′-GCAGGCTAAGGGGGCTCAGC -3′	J, K, M, N, O	152-128	Binns *et al.* (1995)
ASB2	(F) 5′-CCACTAAGTGTCGTTTCAGAAGG -3′ (R) 5′-CACAACTGAGTTCTCTGATAGG -3′	B, K, M, N, O, P, Q, R	256-222	Breen *et al.* (1997)
ASB17	(F) 5′-GAGGGCGGTACCTTTGTACC -3 (R) 5′-ACCAGTCAGGATCTCCACCG -3	G, H, M, N, O, P, Q, R, S	131-89	Breen *et al.* (1997)
ASB23	(F) 5′-GCAAGGATGAAGAGGGCAGC -3′ (R) 5′-CTGGTGGGTTAGATGAGAAGTC -3′	I, J, K, L, S, U	212-176	Irvin *et al.* (1998)
CA425	(F) 5′-AGCTGCCTCGTTAATTCA -3′ (R) 5′-CTCATGTCCGCTTGTCTC -3′	I, J, K, L, M, N, O	250-230	Eggleston-Stott *et al.* (1997)
HMS1	(F) 5′-CATCACTCTTCATGTCTGCTTGG -3′ (R) 5′-TTGACATAAATGCTTATCCTATGGC -3′	I, J, M	178-166	Guerin *et al.* (1994)
HMS3	(F) 5′-CCAACTCTTTGTCACATAACAAGA -3′ (R) 5′-CCATCCTCACTTTTTCACTTTGTT -3′	I, K, M, N, O, P, R	174-150	Guerin *et al.* (1994)
HMS6	(F) 5′-GAAGCTGCCAGTATTCAACCATTG -3′ (R) 5′-CTCCATCTTGTGAAGTGTAACTCA -3′	K, L, M, O, P, R	171-153	Guerin *et al.* (1994)
HMS7	(F) 5′-CAGGAAACTCATGTTGATACCATC -3′ (R) 5′-TGTTGTTGAAACATACCTTGACTGT -3′	J, K, L, M, N, O	189-167	Guerin *et al.* (1994)
HTG4	(F) 5′-CTATCTCAGTCTTGATTGCAGGAC -3′ (R) 5′-CTCCCTCCCTCCCTCTGTTCTC -3′	K, L, M, N, P	141-127	Ellegren *et al.* (1992)
HTG10	(F) 5′-CAATTCCCGCCCCACCCCCGGCA -3′ (R) 5′-TTTTTATTCTGATCTGTCACATTT -3′	I, K, L, M, O, Q, R, S	171-89	Marklund *et al.* (1994)
LEX3	(F) 5′-ACACTCTAACCAGTGCTGAGACT -3′ (R) 5′-GAAGGAAAAAAAGGAGGAAGAC -3′	F, H, J, L, M, N, O, P	160-137	Coogle *et al.* (1996)
VHL20	(F) 5′-CAAGTCCTCTTACTTGAAGACTAG -3′ (R) 5′-AACTCAGGGAGAATCTTCCTCAG -3′	I, L, M, N, O	107-89	Van Haeringen *et al.* (1994)

genomic DNA, 2 mM $MgCl_2$, 0.25 μM of each primer, 1 unit of Taq DNA polymerase, 200 μM of the mix of dNTP and standard reaction buffer. The thermal cycling conditions included an initial denaturation at 95°C for 10 min, followed by 30 cycles of 30 sec at 95°C, 30 sec at 60°C and 1 min at 72°C. A final elongation step was carried out at 72°C for 10 min. The PCR products were resolved on a non-denaturing 10% polyacrylamide gel by electrophoresis then the PCR products remaining obtainable with formamide and electrophoresis was carried out on an ABI PRISM 3100 genetic analyzer using the recommended protocols. DNA fragments separated were performed with genotype software Ver. 3.7. The data analysis conducted by Population Genetics software POPGENE and genetic variation was estimated by calculating number of alleles, observed and expected heterozygosity, Polymorphism Information Content (PIC).

RESULTS AND DISCUSSION

In the present study, 3-9 alleles at loci were detect. The means of observed polymorphism at population was 7.01. At this study, the average of heterozygocity was 0.656 and the expected of heterozygocity at this population was 0.697. The polymorphic information contents average 6.41 in population total. The result of Expected Heterozygocity (EHet), Observed Heterozygocity (OHet) and Polymorphic Information Content (PIC) has shown at Table 2. CA425 has shown the maximum of observed and estimated heterozygosity.

Table 2: Expected Heterozygocity (EHet), Observed Heterozygocity (OHet) and Polymorphic Information Content (PIC) of the Iranian Arab horses

Genetic marker	OHet	EHet	PIC
AHT4	0.736	0.730	0.680
AHT5	0.756	0.731	0.691
ASB2	0.804	0.814	0.788
ASB17	0.781	0.766	0.728
ASB23	0.685	0.613	0.608
CA425	0.852	0.831	0.809
HMS1	0.772	0.853	0.748
HMS3	0.779	0.770	0.788
HMS6	0.553	0.551	0.547
HMS7	0.662	0.644	0.659
HTG4	0.645	0.625	0.610
HTG10	0.618	0.622	0.709
LEX3	0.767	0.766	0.766
VHL20	0.559	0.548	0.690

HMS6 has shown the minimum of observed and estimated heterozygosity. Also, the results of DNA typing for parentage testing in the two families are shown in Table 3.

A fast and accurate way to construct a pedigree is by knowing the genotype of parents and progeny. Microsatellite markers are more likely than other methods to detect small differences between populations due to their high levels of allelic variation being able to discriminate in both overall heterozygosity and mean number of alleles (Caballero and Toro, 2002). Therefore in this study, researchers performed a routine DNA typing with fourteen microsatellite markers to determined genotype and pedigree of the Iranian Arabian horse. At 1st horses microsatellites were characterized by

Table 3: Results of parentage testing by fourteen microsatellite loci in Iranian Arab horses

Samples	AHT4	AHT5	ASB2	ASB17	ASB23	CA425	HMS1	HMS3	HMS6	HMS7	HTG4	HTG10	LEX3	VHL20
Case 1														
Sire	K/O	J/K	M/Q	G/S	I/L	M/M	J/M	K/M	K/P	J/M	K/M	M/Q	F/M	L/M
Dam	K/J	K/K	R/O	G/O	L/K	N/O	J/M	R/O	O/P	L/O	M/N	M/L	M/O	M/N
Foal	K/K	K/M	M/O	G/S	L/U	N/O	M/M	M/O	K/P	O/O	K/N	Q/S	F/O	L/M
Case 2														
Sire	K/O	K/M	M/N	Q/N	L/L	M/L	I/M	N/N	K/M	M/N	P/N	I/L	L/M	I/L
Dam	H/O	M/N	N/O	N/O	K/K	N/O	J/M	O/P	M/P	M/O	M/N	M/M	O/O	N/O
Foal	K/J	K/N	M/Q	N/N	L/J	M/L	I/J	N/O	K/P	N/O	N/L	I/L	O/P	I/L

Ellegren *et al.* (1992) and Marklund *et al.* (1994), they were isolated set of (CA)n repeats and demonstrated that is highly polymorphic in horse.

In Iran, parentage verification was conducted in caspian horse using 7 microsatellites markers. In their report the number of alleles per locus varied from 3-4 with mean value of 3.86. The expected heterozygosity was ranged from 0.617-0.741 (mean 0.675) and the total Exclusion Probability (PE) of 7 microstellite loci was 0.973 (Seyedabadi *et al.*, 2006). In other survey to assist in selection schemes, researchers carried out the first genetic characterization of the Spanish Trotter horse. Result of the mentioned report showed the observed heterozygosity for the Spanish Trotters was 0.647±0.037 and the expected heterozygosity was 0.696±0.026 while the average number of alleles per locus was 6.0±0.341, these values being similar to the data published for other horse breeds (Azor *et al.*, 2007).

CONCLUSION

Some breeds of horse have mated together and mixed breed animals produced in Iran. Therefore, this is very difficult that recognize pure breed animals. This study has shown that this markers can used for parentage testing and also individual identification in Arabian horse breeds in Iran and we can recognized the horses that are pure breed. Of course, we suggest that recognized other microsatellite markers for increasing of accuracy.

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