2011 International Conference on Life Science and Technology
IPCBEE vol.3 (2011) © (2011) IACSIT Press, Singapore

Identity testing with fourteen genetic markers of Arabian horses in Charmahal-va-bakhtiari province

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***Abstract*-Nowadays with develop molecular genetics techniques, beings identification and parentage testing is possible using a lot of molecular markers. STR 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 mammifera. The aim of this study is examine the fourteen genetic markers AHT5, AHT4 ASB23, ASB17, ASB2, VHL20, CA425, HMS7, HMS6, HMS3, HMS1, HTG4, HTG10 and LEX3 of Arabian horses and use they in identity testing it species in Chaharmahal-va-bakhtiari province. Blood samples collected from 13 families of Arabian strain in Chaharmahal-va-bakhtiari province area and DNA was extracted. 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, 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 number of alleles per locus varied from 3 to 9 (average 6.36) in arabian horse. The means of observed polymorphism at population was 7.01. The average of heterozygocity at this study was 0.656. The expected of heterozygocity at this population was 0.697. The Polymorphic information contents average 6.41 in population total. Since that those markers in all of the studying horse strain, have various kinds of DNA fragments. Consequently it seems that these fourteen markers can be used as an applicable marker for identifying Arabian horse.**

Keywords- Arabian horse breeds, Microsatellites, Identity testing

I. INTRODUCTION

The Arab breed is one of the most influential horse breeds in the World (1). It is distributed worldwide and has been involved in the formation of many other horse breeds, such as the Thoroughbred (2).

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. The discovery of polymorphism in short tandem repeat (STR or microsatellites) loci and the introduction of polymerase chain reaction (PCR) methodology (3,4) has led to the establishment of extremely powerful "universal" method for individual identification and for parentage control in humans and animals. Microsatellites are valuable genetic markers due to their dense distribution in the genome, great variation, co-dominant inheritance and easy genotyping. In recent years, they have been extensively used inparentage testing, linkage analyses, population genetics and genetic studies (5).

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The aim of this study is examine the fourteen genetic markers AHT5, AHT4 ASB23, ASB17, ASB2, VHL20, CA425, HMS7, HMS6, HMS3, HMS1, HTG4, HTG10 and LEX3 of Arabian horses and use they in identity testing it species in Chaharmahal-va-bakhtiari province.

II. MATERIAL AND METHOD

Blood samples collected from 13 families Arabian strain in Chaharmahal-va-bakhtiari province area. Blood collected in tubes coated with Na2 – EDTA and transferred to lab for DNA extraction. DNA extraction has conducted with use of DNA-kit (Genomic DNA Purification Kit produced by Sina-gen co. of Iran). Agarose gel (1%) electrophoresis and staining by ethidium bromide has used for observation and quality evaluation of DNA.

The Multiplex polymerase chain reaction (PCR) was used for amplification of fourteen markers with the specific primers. 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 genomic DNA, 2mM MgCl2, 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 s at 95°C, 30 s 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) (6).

III. RESULT AND DISCUSSION

The numbers of alleles at loci of this study have 3 to 9. The means of observed polymorphism at population was 7.01. The average of heterozygocity at this study was 0.656. 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. HMS6 has shown the minimum of observed and estimated heterozygosity.

At first horses microsatellites were characterized by Ellegren et al and Marklund et al. (12, 13) they were isolated set of (CA)n repeats and demonstrated that is highly polymorphic in horse. The nine of these markers has presented by The Horse Genetic Committee of ISAG (AHT4, AHT5, ASB2, HMS3, HMS6, HMS7, HTG4, HTG10 and VHL20). (18) Other markers have added for increased of accuracy. The microsatellites markers have used for parentage testing in some countries.

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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TABLE I. PRIMER SEQUENCES USED FOR THE AMPLIFICATION OF MICROSATELLITE LOCI

Locus	Primer sequences (5'–3')	Product Size) bp (	References
AHT4	(F) 5′- AACCGCCTGAGCAAGGAAGT -3′ (R) 5′- GCTCCCAGAGAGTTTACCCT -3′	170–138	(7)
AHT5	(F) 5′- ACGGACACATCCCTGCCTGC -3′ (R) 5′- GCAGGCTAAGGGGGCTCAGC -3′	152–128	(7)
ASB2	(F) 5′- CCACTAAGTGTCGTTTCAGAAGG -3′ (R) 5′- CACAACTGAGTTCTCTGATAGG -3′	256–222	(8)
ASB17	(F) 5′- GAGGGCGGTACCTTTGTACC -3 (R) 5′- ACCAGTCAGGATCTCCACCG -3	89–131	(8)
ASB23	(F) 5′- GCAAGGATGAAGAGGGCAGC -3′ (R) 5′- CTGGTGGGTTAGATGAGAAGTC -3′	212–176	(9)
CA425	(F) 5′- AGCTGCCTCGTTAATTCA -3′ (R) 5′ CTCATGTCCGCTTGTCTC -3′	250–230	(10)
HMS1	(F) 5′- CATCACTCTTCATGTCTGCTTGG -3′ (R) 5′- TTGACATAAATGCTTATCCTATGGC -3′	178–166	(11)
HMS3	(F) 5′- CCAACTCTTTGTCACATAACAAGA -3′ (R) 5′- CCATCCTCACTTTTTCACTTTGTT -3′	174–150	(11)
HMS6	(F) 5′- GAAGCTGCCAGTATTCAACCATTG -3′ (R) 5′- CTCCATCTTGTGAAGTGTAACTCA -3′	171–153	(11)
HMS7	(F) 5′- CAGGAAACTCATGTTGATACCATC -3′ (R) 5′- TGTTGTTGAAACATACCTTGACTGT -3′	189–167	(11)
HTG4	(F) 5′- CTATCTCAGTCTTGATTGCAGGAC -3′ (R) 5′- CTCCCTCCCTCCCTCTGTTCTC -3′	141–127	(12)
HTG10	(F) 5′- CAATTCCCGCCCCACCCCCGGCA -3′ (R) 5′- TTTTTATTCTGATCTGTCACATTT -3′	89–171	(13)
LEX3	(F) 5′- ACACTCTAACCAGTGCTGAGACT -3′ (R) 5′- GAAGGAAAAAAAGGAGGAAGAC -3′	160–137	(14)
VHL20	(F) 5′- CAAGTCCTCTTACTTGAAGACTAG -3′ (R) 5′- AACTCAGGGAGAATCTTCCTCAG -3′	89–107	(15)

TABLE II. THE RESULT OF OBSERVED AND EXPECTED HETEROZYGOCITY AND POLYMORPHIC INFORMATION CONTENT

marker	OHet	EHet	PIC
AHT4	0/736	0/730	0/680
AHT5	./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	0/753	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