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Short Communication

COMPARATIVE MOLECULAR ANALYSIS OF ISSR MARKERS IN ARABIAN HORSE BREEDS

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ABSTRACT

Microsatellites are abundantly present in nuclear genome. They are renowned for their utility in polymorphism analyses. The informativeness of multilocus ISSR (Inter-simple sequence repeats) markers was compared to analyze the Arabian horse breeds (*Equus caballus*) including Middle Eastern Arabian (28) and mixed (hybrid) Arabians (10). They are helpful in the assessment of genetic diversity among horse populations. The marker $(GA)_9C$ gave 18 alleles in electrophoretic agarose gel. The low number of loci were detected with the $(CA)_9G$ ISSR marker. We calculated the Polymorphism Information Content (PIC) for each of the 12 ISSR markers. In total, 136 alleles were obtained with 12 ISSR markers. The allelic frequencies were also calculated which were ranging from 0.2 to 1.The PIC values were ranging 0.07 to 0.48 from lowest to highest respectively. Trinucleotide-based ISSR markers were found to be more conclusive than the dinucleotide ones. The overall average PIC was 0.2994. The polymorphic bands and their proportion were directly proportional to the PIC value. The highest PIC value detected with the trinucleotide marker $(GTG)_7C$ i.e.(0.48).

Keywords: *Equus caballus,* Inter simple sequence repeats, Genetic diversity, Polymorphism information content (PIC).

INTRODUCTION

Recent studies focus on genetic analysis of different horse breeds, especially the breeds with commercial value, in order to enhance their breeding efficiency and to maintain the genetic diversity (Lindgren, 2001). It is very essential to characterize the genetic structure of the population for breeding, conservation and to select the desirable traits (Iwanczyk *et al.,* 2006). In Middle Eastern Arabian horse population (Saudi Arabia, Syria and Iran), the genetic diversity was higher than the Western Arabians (Polish and Shagya) followed by the Western American Arabian population (Khanshour *et al.,* 2013). The breed maintenance plays an important role in breed's purity and similarly outcrossing/mixing leads to heterozygosity. In non-Arabian breeds high heterozygosity values are reported in recent studies (Conant *et al.,* 2012; Van de Goor *et al.,* 2011). The genetic diversity of the non-breed horses decreased as the migration occurred from east to west (Warmuth *et al.,* 2013). To examine genepool differences multilocus microsatellite markers have widely been used in domestic animals (Stolpovskii *et al.,* 2010 a).

Microsatellites are abundantly present in the nuclear genome. They are renowned for their polymorphism analyses (Aberle *et al.,* 2004). DNA fingerprinting using PCR primers complementary to microsatellites offers a simple approach to study DNA polymorphism in wide range of eukaryotes with potential applications in molecular taxonomy, genome mapping and population genetics (Zietkiewiez *et al.,* 1994). The ISSR markers are single stranded, complementary to the microsatellite sequences and usually 4-12 repeat units. They amplify the unique DNA sequences in-between the inverted microsatellite repeats also known as ISSR fingerprinting.

These markers are widely used in plant genetics and infrequently in animal studies, although they have been informative in animal genetics and diversity studies (Gorodnaya and Glazko, 2003; Kol and Lazebnyi, 2006; Velu *et al.,* 2008). They are also useful in conservation programs which enable to reduce the inbreeding and genetic diversity (Bigi and Perrota, 2012). Furthermore, microsatellite markers used to study the genetic diversity in Criolloand Marismeno horse population recently (Cortes *et al*., 2017; Montserrat *et al*., 2017). In cattle and other ungulates, dinucleotide-based ISSR primers have been used extensively (Stolpovskii *et al.,* 2010 a; Gorodnaya and Glazko, 2003; Bento *et al.,* 2008; Glazko *et al.,* 1999; Stolpovskii *et al.,* 2010 b; Voronkova *et al.,* 2011). However, trinucleotide-based ISSR primers have been more informative than the dinucleotide primers (Voronkova *et al.,* 2011).

The aim of the present work was to analyze the efficiency of ISSR markers to evaluate polymorphism(s) in nuclear genome of Arabian horse breeds.

MATERIALS AND METHODS

Sample collection and genomic DNA extraction: All experimental procedures were reviewed and approved by the Animal Research Ethics Committee of the King Abdulaziz University (Reference No. 298-14 Animal study, 10 November 2014). Blood samples were collected from 38 horses of Middle Eastern Arabian (28) and mixed (hybrid) Arabians (10) from private farms of Jeddah, Saudi Arabia. Blood was taken from the jugular vein and collected in labelled EDTA tubes, which were kept on ice until storage in lab at -20°C. DNA was isolated from 0.2ml blood samples by QIAGEN DNA (Cat. No. 51104; Hilden, Germany) extraction kit, according to manufacturer's protocol. DNA was quantified spectrophotometrically and used for polymerase chain reaction (PCR). ISSR-PCR markers used to analyze polymorphism of DNA fragments flanked by inverted repeats of microsatellite loci based upon the standard protocol (Zietkiewiez *et al.,* 1994).

PCR amplification: Primers used for gene amplification were synthesized at Macrogen Inc, Korea. PCR was carried out in a 25µl reaction mixture containing; 5µL Jena Bioscience *Taq* PCR Master Mix (*Taq* DNA Polymerase, PCR Buffer, $MgCl_2$, and dNTPs), 1µL DNA template of 100ng, 10 picomoles as a final concentration of each primer and distilled water to final volume of 25µL.

The analysis was performed by the traditional ISSR-PCR method according to the standard procedures (Zietkiewiez *et al.,* 1994). The ISSR markers used in the present study are listed in Table 1.

Table 1. Di- and trinucleotide ISSR microsatellite markers along with their annealing temperature.

S.No	Marker	Annealing temperature, °C
1	(GA)9C	51
2	(AG)9C	51
3	(CA)9G	51
4	(GAG)6C	57
5	(ACC)6G	62
6	(CAC)6G	57
7	(AGC)6G	58
8	(CTC)6A	56
9	(CAC)6A	56
10	(CTC)6C	56
11	(GTG)7C	62
12	(CAC)7T	60

Data analysis: Each marker provided reproducible ISSR bands scored as present (1) and absent (0). The performance of the used molecular markers were assessed by using polymorphic information content (PIC). The polymorphism index for each ISSR marker was calculated through summation of the individual PIC as proposed by Roldan-Ruiz *et al.,* (2010). The formula used was as follows

$$PIC_i = 2f_i (1 - f_i)$$

PICi - PIC of the locus I, fi - frequency of the amplified fragments (band present), and (1 - fi) - frequency of non-amplified fragments (band absent). The frequency was calculated as the ratio between the number of amplified bands at each locus and the total number of accessions.

RESULTS AND DISCUSSION

The isolated DNA was used for the amplification of 12 different ISSR markers including three of dinucleotide and nine of trinucleotide markers at varied annealing temperature conditions. The amplified DNA fragments were observed in-between 250bp to 2500bp of various lengths as shown in Fig. 1. The amplified fragments of different length ranging from 50bp-2500bp illustrated in Table 2. The highest number of amplified fragments obtained with $(GA)_9C$ marker denoting highly polymorphic ISSR marker and useful in genetic analysis.

The ISSR markers revealed rational polymorphic loci and PIC value which served as a polymorphism unit as well as gene mapping tool for genetic diversity analyses (Mehta *et al.,* 2007) as shown in the Table 3. Hence, they are reliable for evaluating polymorphism analyses and to differentiate between closely related and isolated species (Stolpovsky *et al.,* 2014). The data provided by these markers were cost-effective and put light on basic level which further useful in analyses at genotypic and sequencing level.

In dinucleotide ISSR markers, the least number of polymorphic loci and PIC values were obtained with $(CA)_9G$. Similarly, in trinucleotide ISSR markers, $(CTC)_6C$ and $(CTC)_6A$ were demonstrated with low number of polymorphic loci which reflected in low PIC (Voronkova *et al.,* 2011). Hence, they are not optimal in genetic analysis of horse breeds.

In Total, 136 different microsatellite loci observed with the average of 11.33 bands per marker. The total number of polymorphic alleles detected were 99, with the average of 8.25 bands per marker. The total average polymorphism proportion was 71.45%. Calculated the PIC values for each primer and average PIC value detected was 0.2994. Highest PIC value obtained for the marker $(GTG)_7C$ (0.48) followed by $(GA)_9C$ (0.44) and the lowest value detected with $(CTC)_6C$ (0.0727). Our PIC values were comparable with one of the recent study on microsatellites (PIC ranging from 0.7- 0.3) (Criscione *et al*., 2015). Results indicated that the PIC value increased with the increase in

polymorphic bands of the particular marker and simultaneously when the polymorphic bands decreases, the PIC value of that particular marker decreased. Hence, the PIC value directly proportional to the number of the polymorphic bands.

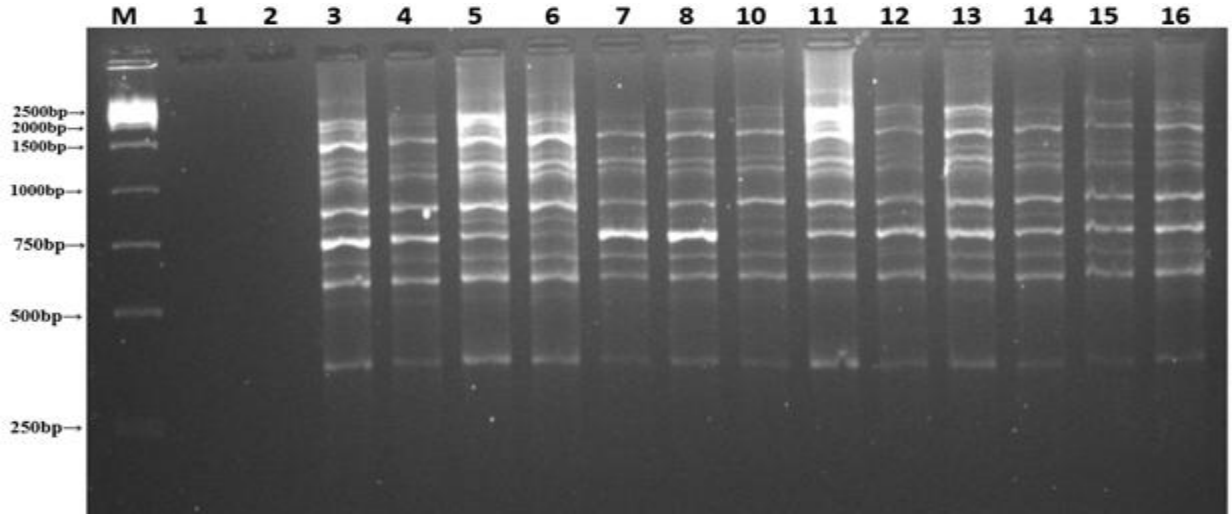

Figure1. The agarose gel picture of ISSR marker PCR with 16 Arabian horse samples. Lane 1 is 1kb marker and 1-16 are samples represented with amplified DNA fragments of different lengths. 1 and 2 samples were not amplified.

Table 2. Distribution of fragments of different lengths and their total amplicons.

S.No	Primer Sequence	Product length (bp)	Fragments	Total amplicons
1	(GA)9C	250-500	4	18
		500-750	3	
		750-1000	4	
		1000-1500	5	
		1500-2500	2	
2	(AG)9C	250-500	1	10
		500-750	2	
		750-1000	1	
		1000-1500	5	
		1500-2000	1	
3	(CA)9G	250-500	3	6
		500-750	1	
		750-1000	1	
		1000-2000	1	
4	(GAG)6C	250-500	2	14
		500-750	3	
		750-1000	3	
		1000-1500	4	
		1500-2000	2	
5	(ACC)6G	250-500	3	15
		500-750	3	
		750-1000	3	
		1000-1500	4	
		1500-2500	2	
6	(CAC)6G	250-500	2	10
		500-750	1	
		750-1000	4	
		1000-1500	3	

7	(AGC)6G	250-500	2	10
		500-750	2	
		750-1000	2	
		1000-1500	2	
		1500-2000	2	
8	(CTC)6A	250-500	1	13
		500-750	4	
		750-1000	2	
		1000-1500	3	
		1500-2000	1	
		2000-2000	2	
9	(CAC)6A	50-250	2	12
		250-500	4	
		500-750	2	
		750-1000	4	
10	(CTC)6C	250-500	1	12
		500-750	2	
		750-1000	3	
		1000-1500	3	
		1500-2000	3	
11	(GTG)7C	250-500	2	10
		500-750	2	
		750-1000	3	
		1000-1500	2	
		1500-2500	1	
12	(CAC)7T	250-500	1	6
		500-750	2	
		750-1000	2	
		1000-1500	1	

Table 3. The 12 ISSR markers and their characteristics.

Marker	Total No. of bands	No. of polymorphic bands	Polymorphic bands (%)	PIC
(GA)9C	18	17	94.44	0.4444
(AG)9C	10	6	60	0.282
(CA)9G	6	1	16.66	0.0833
(GAG)6C	14	13	92.85	0.3385
(ACC)6G	15	9	60	0.3513
(CAC)6G	10	10	100	0.423
(AGC)6G	10	7	70	0.25
(CTC)6A	13	5	38.46	0.1353
(CAC)6A	12	12	100	0.3866
(CTC)6C	12	3	25	0.0727
(GTG)7C	10	10	100	0.48
(CAC)7T	6	6	100	0.3466
Total	136	99		
Average	11.33	8.25	71.45	0.2994

In our study, both the dinucleotide and trinucleotide based ISSR markers were evaluated for their suitability in assessing the horse population genetics. Our data revealed that, in dinucleotide primers $(GA)_9C$ and in trinucleotide-based markers $(GTG)_7C$, $(CAC)_6G$, $(CAC)_6A$, $(ACC)_6G$, $(CAC)_7T$, and $(GAG)_6C$ were more optimal for genetic analysis of horse breeds.

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REFERENCES

Aberle, K. S., H. Hamann, C. Drogemuller and O. Distl (2004). Genetic diversity in German draught horse breeds compared with a group of primitive, riding and wild horses by means of microsatellite DNA markers. Anim. Genet. 35(4): 270-277.

Bento, M., H. S. Pereira, M. Rocheta, P. Gustafson, W. Viegas and M. Silva (2008). Polyploidizationas a retraction force in plant genome evolution: sequence rearrangements in Triticale. Plos ONE 3(1): e1402.

Bigi, D., and G. Perrotta (2011).Genetic structure and differentiation of the Italian catria horse. J. of Hered. 103(1): 134-139.

Conant, E. K., R. Juras and E. G. Cothran (2011).A microsatellite analysis of five colonial Spanish horse populations of the Southeastern United States. Anim. Genet. 43(1): 53-62.

Cortes, O., S. Dunner, L. T. Gama, A. M. Martínez, J. V. Delgado, C. Ginja, L. M. Jimenez, J. Jordana, C. Luis, M. M. Oom and D.P. Sponenberg.(2017). The legacy of Columbus in American horse populations assessed by microsatellite markers. J. of Anim. Breed. Genet.1–11.

Criscione, A., V. Moltisanti, L. Chies, D. Marletta and S. Bordonaro (2015). A genetic analysis of the Italian Salernitano horse. Animal. 9(10): 1610-1616.

Glazko, V. I., T. N. Dyman, S. I. Tarasiuk, and A. V. Dubin (1998).The polymorphism of proteins, RAPD-PCR and ISSR-PCR markers in European and American bison and cattle. TSitol Genet. 33(6): 30-39.

Gorodnaya, A.V., and V.I. Glazko (2003). ISSR-PCR in differentiation of cattle breeds gene pools. Tsitol Genet 39: 61.

Iwańczyk, E., R. Juras, G. Cholewiński and E. G. Cothran (2006). Genetic structure and phylogenetic relationships of the Polish heavy horse. J. of Appl. Genet. 47(4): 353-359.

Khanshour, A., E. Conant, R. Juras and E. G. Cothran (2013). Microsatellite analysis of genetic diversity and population structure of Arabian horse populations. J. of Hered. 104(3): 386-398.

Kol, N. V., and O. E. Lazebny (2006). Polymorphism of ISSR-PCR markers in Tuvinian population of reindeer rangifertarandus L. Russian J. of Genet. 42(12): 1464-1466.

Lindgren, G. (2001). Genome mapping of the horse. Ph.D. thesis, Uppsala University, Sweden.

Mehta, S. C., A. Goyal and M. S. Sahani (2007).Microsatellite markers for genetic characterisation of Kachchhi camel. Ind. J. of Biotech. 6: 336-339.

Montserrat, P. G., V. Landi, A. M. G. Carpio, S. N. Baena, J. V. D. Bermejo, M. D. M. Oom, C. Luis, L. Ouraghand J. L. Vega-Pla (2017). Genetic diversity of the semi-feral Marismeno horse breed assessed with microsatellites. Italian J. of Anim. Sci. 16(1): 14-21.

Roldán-Ruiz, I., J. Dendauw, E. Van Bockstaele, A. Depicker and M. D. Loose (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium spp*.). Mol. Breed. 6(2): 125-134.

Stolpovskii, Yu. A., O. E. Lazebny, K. Yu. Stolpovskii and G. E. Sulimova (2010 a). The use of the ISSR-PCR method for identifying domesticated animal breeds and species, inferring their population structures, and assessing gene pool similarity. Russ. J. of Genet. 46(6): 732-739.

Stolpovsky, Yu. A., N. V. Kol, A. N. Evsyukov, M. N. Ruzina, L. V. Shimiit and G. E. Sulimova (2010 b). Analysis of the genetic structure of Tuvinian short-fat-tailed sheep populations with the use of the ISSR-PCR method. Russ. J. of Genet. 46(12): 1462-1470.

Stolpovsky, Yu A., N. V. Kol, A. N. Evsyukov, L. V. Nesteruk, Ch M. Dorzhu, TsTsendsuren and G. E. Sulimova (2014). Comparative analysis of ISSR marker polymorphism in populations of yak (*Bosmutus*) and in F1 hybrids between yak and cattle in the Sayan-Altai region. Russ. J. of Genet. 50(10): 1025-1037.

Van de Goor, L. H. P., W. A. van Haeringen and J. A. Lenstra (2011).Population studies of 17 equine STR for forensic and phylogenetic analysis. Anim. Genet. 42(6): 627-633.

Velu, D. K., M. Ponnuvel, M. Muthulakshmi, R. K. Sinha and S. M.H. Qadri (2008).Analysis of genetic relationship in mutant silkworm strains of *Bombyxmori* using inter simple sequence repeat (ISSR) markers. J. of Genet. And Genom. 35(5): 291-297.

Voronkova, V. N., T. Tsedev and G. E. Sulimova (2011). Comparative analysis of the informativeness of ISSR markers for estimating genetic diversity of horse breeds. Russ. J. of Genet. 47(8): 1004-1007.

Warmuth, V., A. Manica, A. Eriksson, G. Barker and M. Bower (2012). Autosomal genetic diversity in non-breed horses from eastern Eurasia provides insights into historical population movements. Anim. Genet. 44(1): 53-61.

Zietkiewicz, E., A. Rafalski and D.Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 20(2): 176-183.