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Full Length Research Paper

Detection of genetic variation in sample of Iranian proofed Holstein cattle by using microsatellite marker

Mohammad Reza Movahedin[1], Cyrus Amirinia[2]*, Alireza Noshary[1] and S. Ahmad Mirhadi[2]

[1]Department of Animal Science, Karaj Branch, Islamic Azad University, Karaj, Mehr Shahr, Iran.
[2]Department of Biotechnology, Animal Science Research Institute of Iran, Karaj, Iran.

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This study describes genetic variation among samples of Iranian Holstein cattle (*Bos taurus*) by using microsatellite markers. Semen samples of individuals were taken followed by DNA extraction. A panel of 13 microsatellites was used for evaluation of 13 loci in 68 Holstein proofed bulls. Mean value for allele per locus detected is 6.615, ranging from 10 (SPS115) to 4 (ETH3). All the microsatellite DNA markers showed high polymorphism and displayed a relatively high level of genetic variation as estimated by allelic diversity and heterozygosity. Estimated heterozygosities ranged from 1.000 (BM2113, TGLA122, TGLA126, ETH3, MGTG4B, SPS115, TGLA227 and INRAO23) to 0.633 (SPS113) with mean value of 0.946. All the loci showed deviation from Hardy- Weinberg equilibrium ($p < 0.001$), polymorphism information content (PIC) calculated for each marker exceeded 0.6 and the mean value of Shannon information index was estimated to be 1.606. Obtained results showing heterozygousity can be useful for the development of breeding strategies for genetic improvement in Iranian Holstein cattle.

Key words: Genetic variability, polymorphism, microsatellite loci, *Bos taurus*, Iranian Holstein cattle, DNA.

INTRODUCTION

Livestock industry requires the development of very standard cattle population to fulfill its commercial needs by selection practices in breeding programs. Genetic diversity found in population allows farmers to develop new characteristics in response to changes in environment, diseases, or market conditions (Maudet et al., 2002). The extensive selection and multiplication of superior animals cause a significant decrease in the genetic variation needed for the improvement of economic traits and breeds (Machado et al., 2003). Genetic diversity data also can be used to monitor the genetic structure of populations and detect changes in the frequency of genes due to breeding programs, which makes it possible to preserve the biological diversity of farm animals (Moazami-Goudarzi et al., 1997; Martín-Burriel et al., 1999; Maudet et al., 2002).

Microsatellites have proved to be useful polymorphic markers for the analysis of genetic diversity (Guolizhou et al., 2005). There are more than a thousand cattle microsatellite markers to choose from (Barendse et al., 1994; Kappes et al., 1997). The usefulness of microsatellite markers for the estimation of genetic diversity has been documented in numerous studies (Buchanan et al., 1994; Saitbekova et al., 1999; Schmid et al., 1999; Maudet et al., 2002).

This study was conducted to determine the levels of genetic variation by examining microsatellite DNA polymorphisms among 68 individual Iranian Holstein proofed bulls and 13 microsatellite loci were chosen according to the microsatellites panel recommended by FAO (2004), and also to determine the efficiency of microsatellite markers in estimation of levels of genetic diversity.

MATERIALS AND METHODS

Sampling

Semen samples of 68 progeny- tested and proofed individuals were collected from Animal Breeding Center of Iran (Karaj, Iran) and delivered to the laboratory within liquid nitrogen. Genomic DNA from semen samples were extracted using protocols as previously described by Zadworny and Kuhenlein (1990).

*Corresponding author. E-mail: amirinia@asri.ir Tel/fax: +982614430010

Table 1. Microsatellite primer sequence and annealing temperature.

Locus	Primer sequence	Annealing temperature (°C)	Allele size (bp)
BM1824	GAG CAA GGT GTT TTT CCA ATC CAT TCT CCA ACT GCT TCC TTG	56	176 - 207
BM2113	GCT GCC TTC TAC CAA ATA CCC CTT CCT GAG AGA AGC AAC ACC	56	126 - 150
INRA023	GAG TAG AGC TAC AAG ATA AAC TTC TAA CTA CAG GGT GTT AGA TGA ACT C	59	199 - 241
SPS115	AAA GTG ACA CAA CAG CTT CTC CAG AAC GAG TGT CCT AGT TTG GCT GTG	57	226 - 288
TGLA122	CCC TCC TCC AGG TAA ATC AGC AATCACATGGCAAATAAGTACATAC	58	141 - 163
TGLA126	CTA ATT TAG AAT GAG AGA GGC TTC T TTG GTC TCT ATT CTC TGA ATA TTC C	56	116 - 130
TGLA227	CGA ATT CCA AAT CTG TTA ATT TGC T ACA GAC AGA AAC TCA ATG AAA GCA	56	86 - 115
ETH10	GTT CAG GAC TGG CCC TGC TAA CA CCT CCA GCC CAC TTT CTC TTC TC	55	198 - 238
ETH225	GAT CAC CTT GCC ACT ATT TCC T ACA TGA CAG CCA GCT GCT ACT	57	134 - 174
MGTG4B	GAGCAGCTTCTTTCTTTCTCATCTT GCTCTTGGAAGCTTATTGTATAAAG	51	127 - 155
SPS113	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	55	132 - 170
ETH3	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	54	94-110
TGLA53	CAGCAGACAGCTGCAAGAGTTAGC CTTTCAGAAATAGTTTGCATTCATGCAG	56	161 - 187

Microsatellite amplifications

Selected panel of 13 microsatellites (Table 1) were amplified independently in polymerase chain reaction (PCR), amplification of genomic segments followed the procedure described by Bishop et al. (1994) with alteration of reaction size. All the PCR amplifications were performed in a 25 µl reaction containing 2 µl of the extracted DNA (150 ng), 10 mM-Tris-HCl (pH 8.3), 50 mM-KCl, 1.5 mM-$MgCl_2$, 25 µM of each dNTP, 5 ng of bovine serum albumin (BSA), 1 U of Taq polymerase and 0.1 to 0.5 µM of each primers . The amplification was carried out in a thermocycler (Eppendorf gradient Master cycler) using the following conditions: an initial denaturation step at 95°C for 3 min followed by 35 cycles of 95°C for 30 s, 48, 53, 55 and 58 °C (specific annealing temperature for each primer in Table 1) for 30 s and 72°C for 60 s. Allele sizes were approximated by comparing with DNA Molecular Weight Marker 8 (19 to 1114 bp) Roche Applied Science (Cat. No. 11 336 045 001).

Statistical analysis

Calculation of sample size and maximum number of individuals (n), observed number of alleles (na), effective number of alleles (ne), (Nei's 1973), genetic diversity as observed heterozygosity (H_o), the expected heterozygosity (H_e), shannon's information index (I), test and heterozygote deficiency and evaluation of population structure (F_{ST}) performed using GenAlex version 6 package (Peakall and Smouse, 2006), tests for deviations from Hardy-Weinberg equilibrium (HWE) according to Falconer and Mackay (1996) and possible deviations of genotype frequencies from their expectations were analyzed using chi-square (χ^2) performed by GenAlex version 6. Polymorphic information content (PIC) was estimated using PIC version 1.51 package (ott, 1992).

RESULTS AND DISCUSSION

Microsatellite markers

Overall, 86 alleles were observed from the 13 loci analyzed. All the loci were polymorphic. The number of alleles per locus ranged from 4 (ETH3) to 10 (SPS115) with a mean of 6.615. PIC parameter indicative of the degree of informativeness of a marker for microsatellite markers (Guolizhou et al., 2005) varied from 0.632 (ETH3) to 0.863 (SPS115), PIC value for each marker exceeded 0.6. According to standard selection of microsatellite loci (Barker, 1994), it has been suggested that microsatellite ought to have at least four alleles to be useful for the evaluation of genetic diversity, in the present study, all of 13 microsatellite loci used are considered to be useful for the evaluation of genetic diversity in cattle breeds. Number of alleles, PIC and effective

Table 2. Number of alleles, PIC and effective number of alleles (Ne).

Locus	Na	Ne	PIC
BM1824	6	4.526	0.684
BM2113	6	4.193	0.724
INRA023	7	5.626	0.798
SPS115	10	8.123	0.863
TGLA122	6	4.03	0.711
TGLA126	6	3.744	0.687
TGLA227	6	4.349	0.732
ETH10	6	4.587	0.749
ETH225	9	5.626	0.799
MGTG4B	7	5.063	0.773
SPS113	6	3.347	0.653
ETH3	4	3.245	0.632
TGLA53	7	3.482	0.664
Mean	6.615	4.611	0.728

PIC, Polymorphism information content.

Table 3. Hardy-Weinberg equilibrium.

Locus	df	Chi Sq (χ^2)	Significance level
BM1824	15	57.730	ns.
BM2113	15	72.421	ns.
INRA023	21	68.814	ns.
SPS115	45	131.474	ns.
TGLA122	15	49.394	ns.
TGLA126	15	43.949	ns.
TGLA227	15	37.635	ns.
ETH10	15	73.856	ns.
ETH225	36	126.747	ns.
MGTG4B	21	93.634	ns.
SPS113	15	94.708	ns.
ETH3	6	46.103	ns.
TGLA53	21	65.653	ns.

ns. = Non significant; df, degree of freedom; Chi Sq (χ^2), Chi square test value; P = 0.001.

number of alleles for sample population of Iranian proofed Holstein are given in Table 2.

Genetic diversity

Heterozygosity is a prevalent index for evaluating allelic diversity for genetic markers, values of observed heterozygosity (H_o) and expected heterozygosity (H_e) in each locus which could be considered as a measure for genetic diversity within population. Expected heterozygosity (H_e) across the surveyed population varied between 0.692 (ETH3) and 0.877 (SPS115) (Table 4). Obtained values are in the range of values observed in relative studies (Radko et al., 2005), observed heterozygosity (H_o) ranged from 0.633 (ETH3) to 1 (for 9 loci) and prevalence of fully heterozygote loci could be relevant to small size of studied sample population (68 cattle). 12 loci (H_o) showed greater value than (H_e) excepted for locus SPS113 which demonstrate decrease in heterozygosity. Greater values of H_o in comparison with H_e could be product of gene entrance from outer source into population of study, which is expected in present population due to application of artificial insemination (Machugh et al., 1997). Mean value of 0.94 for observed heterozygosity and range of 5.6 to 6.8 for expected heterozygosity have been reported (Maudet et al., 2002) in relative studies.

The main reason for high level of observed heterozygosity could be because of one of the properties for economical population in a breeding program under intense selection in comparison with native populations. According to Table 3, all loci have shown deviation from Hardy-Weinberg equilibrium ($P < 0.001$), same results have been reported in previous relative studies (Vallejo et al., 2003). There are several reasons why populations may deviate from Hardy-Weinberg equilibrium. Selection either natural or artificial, artificial insemination, population size and breeding programs with intention of maintaining and increasing allelic diversity will change a population's gene frequencies (Russell et al., 2000) and cause the population deviate from Hardy-Weinberg equilibrium.

Values estimated for Shanon information index (I) as more reliable and practical measure for heterozygosity within single population studies, varied from 1.244 (ETH3) to 2.159 (SPS115) with mean value of 1.606 for all loci. Table 4 presents estimated values of observed and expected heterozogosities, Shanon information index (I) and Wright's fixation index (F).

Increment of heterozygote alleles ratio in comparison with homozygote alleles could be another effective factor of deviation from Hardy-Weinberg equilibrium which is approved by negative values of Wright's fixation index (F) presented in Table 4. F_{is} index presents increase or decrease in frequency of observed heterozygosity toward expected heterozygosity ranging from -1 to 1 and 0 which is in Hardy-Weinberg equilibrium when both frequencies are equal. Negative values of F_{is} could also suggest that mating system of the population of study is a managed and non-accidental mating strategy. Mean value of -0.255 for Wright's fixation index could suggest an increment in the observed heterozygosity along with increase in genetic diversity and lack of inbreeding depression.

Conclusion

According to the results, all the microsatellite loci been used in the current study have shown high level of polymorphism in sample population of Iranian proofed Holstein cattle and are proper to be used in future studies as parentage testing and progeny test.

Table 4. Heterozygosity and Shanon information index (I) and Wright's fixation index (F).

Locus	Ho	He	I	F_{is}
BM1824	0.938	0.779	1.593	-0.205
BM2113	1.000	0.761	1.540	-0.313
INRA023	1.000	0.822	1.805	-0.216
SPS115	1.000	0.877	2.159	-0.140
TGLA122	1.000	0.752	1.513	-0.330
TGLA126	1.000	0.733	1.444	-0.364
TGLA227	1.000	0.770	1.558	-0.299
ETH10	0.984	0.782	1.629	-0.258
ETH225	1.000	0.822	1.859	-0.216
MGTG4B	1.000	0.803	1.708	-0.246
SPS113	0.633	0.701	1.389	0.097
ETH3	1.000	0.692	1.244	-0.445
TGLA53	0.984	0.713	1.440	-0.380
Mean	0.964	0.769	1.606	-0.255

Heterozygosity results could be a standard measure and index for in-population genetic diversity which in the current study. Levels of heterozygosity suggest a proper level of genetic diversity in the population despite intense selection which could be applied in breeding programs to reach optimum level of genetic trend and genetic gain also for unpredicted future demands.

REFERENCES

Barendse W, Armitage SM, Kossarek LM, Shalom A, Kirkpatrick BW, Ryan AM, Clayton D, Li L, Neibergs HL, Zhang N, Grosse WM, Weiss J, Creighton P, McCarthy F, Ron M, Teale AJ, Fries R, McGraw RA, Moore SS, Georges M, Soller M, Womack JE, Hetzel DJS (1994). A genetic linkage map of the bovine genome. Nat. Genet. 6(3): p. 227.

Barker JSF (1994). A global protocol for determining genetic distances among domestic livestock breeds. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph and Ontario, Canada, 21: 501-508.

Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SL, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo J, Beattie CW (1994). A genetic linkage map for cattle. Genetics, 136: 619-639.

Buchanan FC, Adams LJ, Littlejohn RP, Maddox JF, Crawford AM (1994). Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics, 22: 397-403.

Falconer DS, Mackay TFC (1996). Introduction to Quantitative genetics.Ed 4th, Longmans Green, Harlow. Essex, UK.

FAO (2004). Secondary guidelines for development of national farm animal genetic resources management planes. Measurement of domestic animal diversity (MoDAD): Recommended microsatellite markers, Rome, Italy.

GuoliZhou H, Qizhu S, Yuho U (2005). Genetic diversity analysis of five cattle breeds native to China using microsatellites. J. Genet. 84: p. 1.

Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TPL, Lopez-Corrales NL, Beattie CW (1997). A second generation linkage map of the bovine genome. Genome Res. 7: 235-249.

MacHugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG (1997). Microsatellite DNA variation and the evolution, domesticationand phylogeography of Taurine and Zebu cattle (*Bos taurus* and *Bos indicus*). Genetics, 146: 1071-1086.

Machado A, Ivan S, Mário Luiz M, Ana Lúcia CR (2003). Genetic Diversity of Four Cattle Breeds Using Microsatellite Markers Bras. Zootec. 32(1): 93-98.

Martín-Burriel I, García-Muro E, Zaragoza P (1999). Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. Anim. Genet. 30: 177-182.

Maudet C, Luikart G, Taberlet P (2002). Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis, J. Anim. Sci. 80: 942-950.

Moazami-Goudarzi K, Laloë D, Furet JP, Grosclaude F (1997). Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. Anim. Genet. 28: 338-345.

Nei M (1973). Analysis of gene diversity in subdivided populations. PNAS, 70: 3321-3323.

Ott J (1992). Strategies for characterizing highly polymorphic markers in human gene mapping. Am. J. Hum. Genet. 51: 283-290.

Russell ND, Rios J, Erosa G, RemMenga MD, Hawkins DE (2000). Genetic differentiation among geograPHically isolated populations of Criollo cattle and their divergence from other *Bos taurus* breeds. J. Anim. Sci. 78: 2314-2322.

Saitbekova N, Gaillard C, Obexer-Ruff G, Dolf G (1999). Genetic diversity in Swiss goat breeds based on microsatellite analysis. Anim. Genet. 30: 36-41

Schmid M, Satbekova N, Gaillard C, Dolf G (1999). Genetic diversity in Swiss cattle breeds. J. Anim. Breed. Genet. 116: 1-8.

Vallejo L, Li YL, Rogers GW, Ashwell MS (2003). Genetic Diversity and Background Linkage Disequilibrium in the North American Holstein Cattle Population, American Dairy Science Association. J. Dairy Sci. 86: 4137-4147.

Zadworny D, Kuhenlein U (1990). The identification of the Kappa-casein genotype in Holstein dairy cattle using the polymerase chain reaction. Theor. Appl. Genet. 80: 631-634.