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Determination of Genetic Polymorphism in Kerman Holstein and Jersey Cattle Population Using ISSR Markers

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Abstract: In this research, we genotyped 120 animal from Holstein breed and 100 animal from jersey breed using (GA)9C-ISSR and (AG)9C-ISSR markers. The genetic variability such as effective number, gene diversities, polymorphism percentage, number of polymorphic DNA fragments and Hardy-Weinberg equilibrium were calculated by POP Gene software. Number of polymorphic loci and polymorphism percentage based on marker were more in jersey breed than Holstein, demonstrating higher polymorphic percentage in Jersey breed. Analysis of Shannon Index and number actual effective also showed that genetic diversity is higher in Jersey breed.

Key words: Holstein breed, jersey breed, , Polymorphism, ISSR marker.

INTRODUCTION

Many molecular marker techniques are available today PCR based approaches are in demand because of their simplicity and requirement for only small quantities of sample DNA, example RAPD, AFLP, SSR, ISSR (Beckmann, J.S., 1987). A marker system called Inter simple sequence Repeats (ISSR) has only recently been developed an anonymous. ISSR are arbitrary multi loci markers production with microsatellite primer. Studies of ISSR heritability have demonstrated an exceedingly close approximation to classic Mendelian ratios (Glazko, V., 2003). The ISSR primers use to generate the variation in given DNA sample include one of these highly variable microsatellite sequences and arbitrary pair of bases at the 3'end (lord,E.A., 1998). One sample for variation among DNA in small PCR reactions using one primer at time. Where the primer successfully locates two microsatellite regions within an amplifiable distance away on DNA strands of DNA sample, the PCR reaction will generate a band of particular size for that "locus" representing the intervening stretch of DNA between the microsatellites (lord,E.A., 1998).

Usually several to many such "paired" microsatellites areas exist in particular DNA sample, so one gets that many bands generated in the reaction, for that sample (Glazko,V.I., 1995). To date, very small number of published papers using ISSR markers have focused on identification or genotyping of variants in agriculturally important plants and animal. In 1995 Glazko et al estimated of polymorphism of protein using ISSR-PCR marker in Europeans American bioson and cattle. It was shown, that the evaluation of interspecies genetic interrelations was connected more with the determined molecular-genetic markers (loci), included in analysis, than with the marker's belonging to certain type (protein polymorphism, variability of DNA repeat distributions (Glazko,V.I., 1995).

In 1998 Glazko and et al evaluated of genetic variability of 23 bovine microsatellite markers in four Belgian cattle breed (V.I. Glazko,). In 2001 Zubets and *et al* investigation molecular and genetic polymorphism in tree cattle breeds System for investigation of molecular-genetically polymorphism in the cattle breeds and characterization of genotype of individual animals were developed and tested on a real population. In 2003 Gorodnaya and Glazko used of ISSR-PCR in differentiation of cattle breed gen pool. The possibility of using ISSR-PCR markers for characterization of genetic relations between breeds of cattle were evaluated (Gorodnaya, A.V., A.V.Glazko 2003). In 2005 Mohammad Abadi and *et al* implicated of ISSR for identification of polymorphism in some native cattle breed (Mohammad Abadi,

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2005). In 2005 Thriapitsyna and Glazko used of ISSR-PCR for investigation polymorphism of DNA in cattle reproduced under low-does irradiation condition. Results showed Increasing of the heterozygosity calculated (He) in F2 generation has been found that may be considered as response to ecological stress factor (Triapitsyna, N.V., 2005)

MATERIAL AND METHODS

Blood sample were collected from 120 animal from Holstein breed in 4 herds (H1, H2, H3, H4) and 100 animal from jersey breed in 5 herd (J1, J2, J3, J4, J5). They were abstained from jugular aseptic venipuncuture into venoject tubes with EDTA. After wards, they were frozen at -20c until required. DNA was extracted from whole blood with Guanidinium Thiacyanate- Silica gel method using of Diatom DNA prep kit. In this paper used of nucleotide primers with (AG)9C and (GA)9C sequencing. Make a standard PCR reaction master mix with one of primers, aliquot 25 micro liters of master mix in individual tubes as usual, put 1-1/5 micro liter of undiluted DNA sample into each tube. And amplify them.
The amplification program: 94c for 2 minutes; 35 cycles of: 94c for 30 secs,55c for 30secs, 72c for 2 minutes: 72cfor 2 minutes: and 4c soak forever.

After amplification were run out 10 micro liters of 25 micro liters products on a 1% agarose mini-gel using electrophoresis 100pb ladder used as a size standard for sizing PCR products, to visualize the products. Just until the tracking dye has traveled 2/3 of gel length to check for good amplification. Gel were stained with ethidium bromide. The gels washed and then genotypes were scared by UVIDOC software.

Results:

A few bands appear and disappear at random. Depending on conditions and probabilistic mature of PCR. Bands are scored as "present" for sample and a given primer only where they occur bothes replicates and "absent" where they accure in only one replicates or neither of them. Each fragment scored as "present" is treated as a dominant(amplified) band for that locus, while one scored as absent treated as a recessive(null) band. Note that homozygous dominant and heterozygous genotypes cannot be distinguished in diploid individuals (Yeh, F.C., 1999).

These are subjected either to a parsimony or other phylogenic analysis, cluster analysis using a simple matching coefficient such as Jaccard's or an estimate of genetic distance(Nei's distance) modified to accommodate dominant markers (Yeh, F.C., 1999). In this paper, we used of POP Gene and NTSYS software for analysis (NTYSIS pc, Yeh, F.C., 1999). Due to ISSR loci have high polymorphism, Shannon Index indicate genetic variety in each population. Results are presented in figure 1&2.

Other factor that indicate genetic diversity is number actual allelic(na) and number effective allelic(ne) in population. Results showed in figure 3&4. Iin this paper number of polymorphic loci and polymorphism percentage calculated. Results showed in figure 5.6.

Table1: Average Shannon Index for each herd with (AG)9C primer

Herd	H1	H2	H3	H4	J1	J2	J3	J4	J5
Average of Shannon index	0/168	./210	./193	./170	./166	./280	./244	./293	./206

Table 2: average Shannon Index for each herd with (GA)9C primer

Herd	H1	H2	H3	H4	J1	J2	J3	J4	J5
Average of Shannon index	./221	./340	./442	./440	./398	./582	./530	./632	./573

Table 3: average of na and ne in each herd for (AG) 9C primer.

Herd	H	J
na	1/4	1/85
ne	1/20	1/27

Table 4: average of na and ne in each herd for (GA)9C primer.

Herd	H	J
na	1/8	2
ne	1/60	1/76

Table 5: number of polymorphic loci and polymorphism percentage in each herd for (AG)9C primer

Herd	H1	H2	H3	H4	J1	J2	J3	J4	J5
Number of loci	8	8	15	10	15	23	18	13	10
Polymorphism%	29/3	29/3	52/7	41/4	52/7	65/7	53/4	50/4	41/4

Table 6: number of polymorphic loci and polymorphism percentage in each herd for (GA)9C primer

Herd	H1	H2	H3	H4	J1	J2	J3	J4	J5
Number of loci	6	5	11	5	12	11	12	9	9
Polymorphism%	50	45/4	91/6	45/4	100	91/6	100	75	75

Discussion:

We found that: (Beckmann,J.S., 1987) the resolving power of agarose gels is poor relative to that provided by sequencing gels; (V. Glazko,2003) fluorescent labeling of ISSR amplification primers produced numerous scorable bands; (Glazko,V.I., 1995) ISSR fingerprinting patterns are highly heritable; and (Glazko,) double priming ISSR is an easy and informative genetic marker system in cattle for revealing both inter- and intraspecific variations. Average of effective number (ne) in breeds showed that in jersey breed is higher. This result indicated genetic diversity in jersey breed was higher than Holstein. polymorphism percentage and number of polymorphic was higher in jersey breed.

REFERENCE

Beckmann, J.S., M. Soller., 1987. Molecular Markers in the genetic improvement of Farm animals. Biotechnology 5: 573.

Glazko,V., 2003. An attempt at understanding the genetic basis of domestication. Animal science. No 2: 109-120

Glazko,V.I., T.N. Dyman., S.I. Tarasiuk, A.V. Dubin., 1995. The polymorphism of protein,RAPD - PCR and ISSR-PCR marker in European American bison and cattle.

Glazko, V.I,T.N. Dyman, S.I. Tarasiuk, A.Dansercoer, g.mammens,. F.Coopman,.y. Bouquet,.a.Burny,.R.Renaville., D.portetelle, 1998.Evaluation of genetic variability of 23 bovine microsatellite markers in four Belgian cattle breed. Anim Genet. 29(3): 161-167.

Gorodnaya, A.V., A.V.Glazko, 2003. ISSR-PCR in Differentiation of cattle breed gene pools. sitologiya and Genetika. 1: 61-67.

lord, E.A., G.H. Davis., K.G.Doods., H.M. Henry, j.M. Lumsden, G.W. Montogomery, 1998. Identification of Booroola carries.

Mohammad Abadi, M.R., T.A. Kovalenko., M.R.Nasiri.G.E. Sulimova, 2005. Inter_simple Sequence Repeat (ISSR-PCR).For the identification of polymorphism in Some native cattle breed. Proceedings of 3rd Moscow international congress BIOTECHNOLOGY. Moscow Russia March, 14-18: 282.

NTYSIS pc, Numerical taxonomy system, version 2.2 for windows 98/NT/2000/xp.

Triapitsyna, N.V., V.I. Glazko, 2005. polymorphism of DNA. Fragments Flanked by microsatellite loci (ISSR-PCR) in Cattle reproduced under low-does irradiation condition -Tisitol Genet.39(5): 41-50.

Yeh, F.C., R. Yang, and T. Boyle, 1999. Pop Gene version 1.31, Microsoft windows-based free ware for population genetic analysis, University of Alberta. Edmonton, AB, Canada.