EFFECT OF DNA MICROSATELLITE MARKERS ON MILK FAT PERCENTAGE OF CROSSBRED CATTLE OF KERALA

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Abstract

A recent application of molecular technology in dairy cattle breeding is the identification of the regions of the DNA affecting the production traits. In the present study, the possibility of using the informations of the allele frequency, heterozygosity and PIC of two microsatellite markers and their association with the economically important traits for the selection of crossbred cattle were studied. Both the markers were highly informative, as their PIC values were more than 0.5. Animals with the allele 205 at HUJII77 locus had significantly lower milk fat percentage compared to the animals without this allele. The selection against this allele may contribute much in improving the milk fat percentage. For BM4305 locus, the allele 154 had effects on lower milk fat percentage. The selection against this allele may contribute much in improving the milk fat percentage. The animals with the allele 166 had the highest average of milk fat percentage. Selection for this allele will have good impact on higher milk fat percentage.

Key words: *Microsatellite markers, Milk Fat Percentage, Heterozygosity.*

The important applications of molecular markers in conventional breeding programmes include linkage mapping of Quantitative Trait Loci (QTL), marker assisted selection (MAS) and marker assisted introgression. MAS is the process of using the results of DNA testing to assist in the selection of individuals to become parents in the next generation. In the present study, possibility of using the information of the allele frequency, heterozygosity and polymorphic information content of two polymorphic microsatellite markers (HUJII77 and BM4305) in the selection of crossbred cattle for milk fat percentage was studied. According to Shalom *et al.* (1994) the microsatellite marker HUJII77 was located on BTA3 with a relative position of 81.331 cM, with a size range of 187-213 bp and this marker has dinucleotide GT repeat sequences, which can be represented as $(GT)_2TT(GT)_{15}$ with 8 alleles and a heterozygosity of 86%. This was also confirmed by Ihara *et al.* (2004) in the genetic map of the cattle genome based on 3802 microsatellites. The microsatellite marker BM4305 is located on BTA14 with a relative position of 83.309 cM and heterozygosity of 69 with a size range of 148-168 bp (Bishop *et al.*, 1994; Ihara *et al.*, 2004). Heyen *et al.* (1999) detected that the same marker affected milk yield also.

Materials and Methods

Blood and milk samples were collected from animals from two dairy cattle farms of the Kerala Agricultural University namely, University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi. DNA samples from 117 animals were used to find out the PIC of the selected markers. DNA

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was extracted by modifications in the phenol-chloroform protocol (Andersson *et al.*, 1986) and milk samples were analysed for fat percentage (IS: 1224, 1977).

Two markers *viz.*, HUJII77 and BM4305 were chosen for the study. The primers for these markers were custom synthesised. The markers were typed for their polymorphism. For visualising the PCR products by autoradiography, forward primer of each marker was radio-labelled at the 5' end with ã^{32}P-ATP. The reaction was carried out with the DNA endlabeling Kit 1 (Genei). For each microsatellite loci PCR conditions were standardised separately.

To determine the allele size of markers, comparison with a sequencing ladder is necessary. Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0 (M/s Amersham Biosciences Corporation, USA). The radio-labeled PCR products were fractionated using 6 per cent denaturing polyacrylamide gels. A volume of 3.5 µl of formamide loading buffer (0.02 per cent Xylene Cyanol, 0.02 per cent Bromophenol Blue, 10 m*M* EDTA, 98 per cent deionised formamide) was added to the PCR products, mixed well, denatured at 95°C for 5 min and cooled immediately on ice. Again a volume of 3.5-4 µl each of this mixture was loaded into each well. Sequenced products of M13 DNA were loaded in four wells (G, A, T, C). After electrophoresis, the gel was dried and autoradiographed. The number of alleles for each marker was counted and their size was determined by comparing with M13 sequencing ladder. The G, A, T and C sequences were read from the bottom to the top in the order. The allele sizes were determined corresponding to the G, A, T and C bands and the allele frequency was worked out.

Heterozygosity was calculated by the method of Ott (1992). The unbiased heterozygosity was calculated using the formula of Pandey *et al.* (2002). PIC values for the markers were calculated (Botstein *et al.*, 1980). Large sample test (Z test) for the comparison of means of allele containing population with that of the population without the allele was done by the method suggested by Snedecor and Cochran (1985).

Results and Discussion

The alleles present in the sires of the cows under study were considered for the analysis. The allelic effects of HUJII77 and BM4305 on milk fat percentage of crossbred dairy cattle of Kerala are presented in the table.

1. HUJII77

Thirteen alleles with a size range of 193-221 bp and 36 genotypes were observed for the microsatellite marker HUJII77 in crossbred cattle of Kerala. Shalom *et al.* (1994) and Ihara *et al.* (2004) reported a size range of 187-213 bp for the marker and the number of alleles observed by them was eleven. For HUJII77, direct count heterozygosity, unbiased heterozygosity and PIC were 0.851, 0.854 and 0.842. This means the marker is highly informative.

The animals with the allele 205 at HUJII77 locus showed a significantly lower milk fat percentage (3.3±0.18), compared to the animals without this allele (3.78±0.11). This microsatellite marker is located on BTA3, in which the markers ILSTS096 and BL41 are located and both of them have strong associations with milk fat percentage (Heyen *et al.*, 1999). This may be the reason for the association shown by the alleles of HUJII77 with the milk fat percentage.

Table. Effects of HUJII77 and BM4305 alleles on milk fat percentage of crossbred dairy cattle of Kerala

Sl.No.	Alleles of BM4305	Average Milk Fat Percentage	Alleles of HUJII77	Average Milk Fat Percentage
1	146	3.94±0.14 ^{b}	203	3.48±0.16 ^{b}
2	148	3.53±0.33 ^{b}	205	3.30±0.18 ^{a}
3	154	3.19±0.23 ^{a}	207	4.00±0.34 ^{b}
4	156	3.60±0.24 ^{b}	209	3.85±1.44 ^{b}
5	158	3.79±0.16 ^{b}	211	3.56±0.22 ^{b}
6	160	3.66±0.14 ^{b}	213	4.00±0.24 ^{b}
7	162	3.68±0.23 ^{b}		
8	166	4.58±0.20 ^{c}		

Means bearing same superscripts do not differ significantly (P<0.05)

2. BM4305

Twelve alleles were detected with a size range of 146-168 bp and 37 genotypes in the genetically unrelated population. For BM4305, direct count heterozygosity, unbiased heterozygosity and PIC were 0.861, 0.864 and 0.846. This means this marker is also highly informative.

The average milk fat percentage was 3.19±0.23, for the animals with the allele 154, which was significantly lower from the animals without this allele (3.81±0.0106).

The allelic average for milk fat percentage is very high (4.58±0.2) for the allele 166. The allele 166 had a frequency of 0.063, which is low in the population. Hence selection for this allele can be advocated strongly for the population under study. It is recommended to undertake a study with larger number of samples and more number of daughters for effective marker assisted selection.

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