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# Genotyping of *β-Lactoglobulin* (*β-Lg*) gene by PCR-RFLP in indigenous cattle of Assam, India

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## ABSTRACT

The study was conducted to investigate the polymorphism in *β-Lactoglobulin* (*β-Lg*) gene in indigenous cattle of Assam. Genomic DNA from 53 indigenous cattle was extracted and used to study the polymorphism in *β-Lg* gene using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. Genetic improvement by selective breeding provides enormous potential to enhance the performance of animals. But, it is a time-consuming exercise as generation interval of cattle is longer. Besides, milk production being a sex limited trait, it is not possible to select male animal on the basis of its own performance. To minimize these problems, selection based on markers can play an important role to carry out genetic improvement of animals before expressing actual target traits. A PCR product of 262 bp obtained upon amplification was subsequently digested with restriction endonuclease *BSuR*I and yielded three types of restriction pattern, two fragments (153 and 109 bp) for AA genotype, three fragments (109, 79 and 74 bp) for BB genotype and, four fragments (153, 109, 79 and 74 bp) for AB genotype. The frequencies of alleles A and B were found to be 0.2547 and 0.7453 and those of AA, AB and BB genotypes were 0.0943, 0.3207 and 0.5849, respectively. From the present study it could be concluded that the *β-Lg* B variant was predominant in the indigenous cattle with the highest frequency of BB homozygote followed by AB heterozygote (BB>AB>AA) and Chi-square ($x^2$) test revealed that the population under study was in Hardy-Weinberg Equilibrium.

**Key words:** *β-Lactoglobulin*, Gene frequency, Genotyping, Indigenous cattle.

## INTRODUCTION

Improvement of efficiency and economic returns is an important goal in dairy farming, as in any agricultural enterprise. Genetic improvement by selective breeding provides enormous potential to enhance the performance of animals. However, as generation interval of cattle is longer and also milk production being a sex limited trait, selective breeding turns out to be a time-consuming exercise. Hence, to minimize these problems, selection based on markers can play an important role to carry out genetic improvement of animals before expressing actual target traits. Marker assisted selection (MAS) gives a new hope to the animal breeders, which when used in conjunction with conventional selection methods can prove to be extremely advantageous (Bhattacharya and Gandhi, 1997). The discovery of PCR-RFLP generated renewed interest in the use of genetic marker loci as an aid to selection programme for improvement of livestock (Soller and Beckmann, 1983). The milk protein genes are excellent candidate genes for linkage analysis with QTL because of their biological signiûcance on the quantitative traits of interest (Moody *et al*., 1996).

*β-Lg* and k-Cn are the most important proteins expressed in milk. The proteins k-Cn and β-Lg are known to possess great effects on milk production and milk constituents (Marziali and Ng-Kwai-Hang, 1986; Grosclaude, 1988, Aleandri *et al*., 1990; Erhardt, 1996). So, if the DNA polymorphisms of *β-Lg* and *k-Cn* genes are associated with milk production traits, they could serve as genetic markers for genetic improvement in future marker-assisted selection programme in indigenous cattle of Assam. Two major genetic variants of *β-Lg*, A and B, were identified (Lum *et al*., 1997). β-lactglobulin is coded by *β-Lg* gene, which encodes the main protein of whey and is located on chromosome 11 of the bovine genome (Eggen and Fries, 1995).

Assam is endowed with rich genetic resources of different livestock and possesses 8.4 million cattle, out of which 7.9 million is native (17th Livestock Census of Assam, 2003). The productivity and reproductivity of the indigenous livestock is poor in comparison to the exotic breeds but they are well adapted to local hot and humid climatic condition. Keeping all these facts in view, the present study was

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undertaken in indigenous cattle of Assam to identify polymorphism in genomic sequences of *β-Lactoglobulin* (*β-Lg*), to characterize new alleles (if any) and to estimate the gene and genotype frequencies.

## MATERIALS AND METHODS

The present study was conducted on indigenous cattle of Assam from three different districts, *viz.*, Darrang, Silchar and Kamrup. Five ml of blood was aseptically collected from fifty three apparently healthy indigenous cattle using EDTA (2.7%) as anticoagulant. The samples were properly labelled and transferred to the laboratory and genomic DNA was extracted immediately (Sambrook and Russell, 2001).

The purity of the genomic DNA was assessed by UV spectrophotometer (Nanodrop Spectrophotometer, Model- UV/VIS 916) by checking the optical density (OD) value at 260 and 280 nm. The samples having OD ratio (260 nm/ 280 nm) 1.7 to 1.9 were used for the experiment.

Horizontal submarine agarose gel electrophoresis (0.8%) was performed to check the integrity of DNA and the DNA was visualized under gel documentation system. The genomic DNA samples having good quality DNA (intact bands without smearing in gel) were used for further analysis.

The primer sequences used for the amplification of *β-Lg* gene were F:5'-GTCCTTGTGCTGGACACCGACT ACA-3' and R:5'-CAGGACACCGGCTCCCGGTATATGA-3' (Medrano and Aguilar-Cordova, 1990). The primers were diluted for working concentration of 10 pmol/ µl. The PCR reaction was carried out in 0.2 ml PCR tubes in a thermal cycler (BIO-RAD Model S100, USA). PCR was carried out in a final reaction volume of 25 µl. Each reaction volume contained DNA template 1.0 µl, forward and reverse primer 0.5 µl each, master mix 12.5 µl and nuclease free water 10.5 µl.

Cycle condition for amplification was, initial denaturation at 95°C for 5 mins, followed by denaturation at 94°C for 1 min, annealing at 65°C for 1 min, extension at 72°C for 30 secs and final extension was performed at 72°C for 5 mins. Thirty five cycles were run to have optimum amplification.

PCR amplification was confirmed by 1.5% agarose gel electrophoresis and amplified product was visualized under gel documentation system. The PCR product was purified using PCR purification kit and digested with *BsuR*I (Thermo Scientific) as per the manufacturer's protocol. Twelve percent (12%) polyacrylamide gel electrophoresis was performed to visualize the band pattern.

The purified PCR products were sent for sequencing to SciGenom Pvt. Ltd., Cochin, Kerala. Allele and genotypic frequencies were calculated using POPGENE 2.0. Chi-square ($x^2$) test was performed to know if the population was in Hardy-Weinberg equilibrium.

## RESULTS AND DISCUSSION

The amplification of *β-Lg* gene yielded a product size of 262 bp (Fig.1) in all the samples of indigenous cattle of Assam used in the present study and was in good agreement with the results reported by Sitkowska *et al*. (2009) in 155 cows bred in the Kujavian-Pomeranian voivodeship. Similar findings were also obtained by Remus-Alexandru *et al*. (2010) in Romanian Grey Steppe cattle, Gouda *et al*. (2011) in Egyptian cattle and buffalo and Oner *et al*. (2011) in Brown Swiss and Holstein breeds reared in Bursa Region.

Indigenous cattle of Assam was screened to detect polymorphism in the amplified segment of *β-Lg* gene. The enzyme-digested products were subjected to PAGE alongwith 50 bp ladder. The bands were analyzed by comparing with molecular size ladder. Restriction digestion analysis of 262 bp PCR products of *β-Lg* gene with *BSuR*I revealed three types of fragment pattern (Fig.2), arbitrarily designated as AA, AB and BB genotypes. AA genotype revealed two fragments of 153 bp and 109 bp, AB genotype yielded four fragments of 153 bp, 109 bp, 79 bp and 74 bp, while BB genotype showed a restriction pattern of three fragments 109 bp, 79 bp and 74 bp in indigenous catle of Assam. This finding was in conformity with earlier observations of Remus-Alexandru *et al*. (2010) in Romanian Grey Steppe cattle, Gouda *et al*. (2011) in Egyptian cattle and, Oner *et al*. (2011) in Brown Swiss and Holstein breeds reared in Bursa Region. The gene and genotypic frequencies of AA, AB and BB are presented in the Table 1.

The sequence obtained was analyzed by BLAST and submitted to NCBI. In sequence of *β-Lg* gene, one *BSuR*I restriction site was found for AA genotype, three for AB

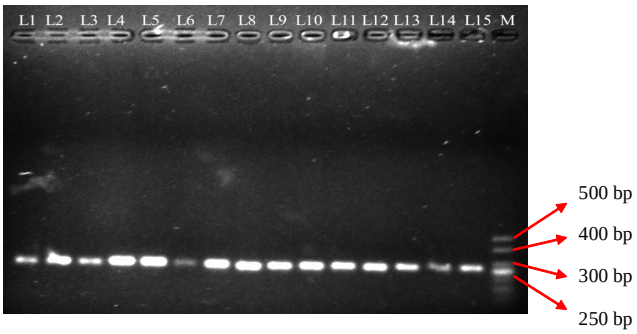


**Fig 1:** PCR amplicon (262 bp) of *β-Lg* gene in 1.5% Agarose gel L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14, L15 : PCR product M : 50 bp ladder

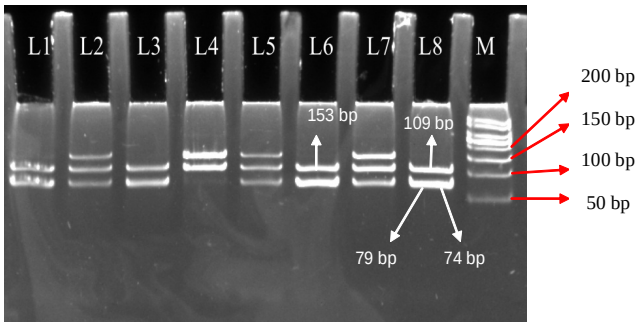


**Fig 2:** PCR- RFLP of *β-Lg* gene in 12% non-denaturing PAGE
L1, L3, L6 and L8 : BB genotype (109, 79 and 74 bp)
L4 : AA genotype (153 and 109 bp)
L2, L5 and L7 : AB genotype (153, 109, 79 and 74 bp)
M : 50 bp ladder

**Table 1:** The distribution of *β-Lg* genotypes, their allele frequencies in indigenous cattle of Assam and Hardy-Weinberg Equilibrium

| LOCI | | GENOTYPE | | | ALLELE FREQUENCY | |
|---|---|---|---|---|---|---|
| | | AA | AB | BB | A | B |
| *β –Lg* | Observed Frequency | 5 | 17 | 31 | 0.2547 | 0.7453 |
| | Expected Frequency | 3.3429 | 20.3143 | 29.3429 | | |
| | Genotype frequency | 0.0943 | 0.3207 | 0.5849 | | |
| | $\chi^2$ | | $1.4525^{N.S}$ | | | |

N.S.= Non significant

genotype, and two restriction sites for BB genotype. All the sequences of *β-Lg* gene showed 99-100% similarity with the other *Bos indicus* and *Bos taurus* sequences.

The allelic and genotypic frequencies were calculated using POPGENE 2.0. The frequencies of A and B alleles of *β-Lg* gene were 0.2547 and 0.7453 and, those of AA, AB and BB genotypes were 0.0943, 0.3207 and 0.5849, respectively.

The present findings revealed a higher frequency of BB genotype followed by AB genotype for *β-Lg* gene in indigenous cattle of Assam. Frequency of AA genotype was the lowest. These data were in conformity with those of Del Lama and Zago (1996), Badola *et al.* (2004), Rachagani *et al.* (2006), Patel *et al.* (2007) and Karimi *et al.* (2009) who also reported the highest frequency of BB genotype in Indian cattle. Likewise, BB genotype was the most frequent for Iranian native cattle (Doosti *et al.* 2011) and also among Chinese Holstein and Jersey (Ren *et al.*, 2011). However, there were reports, where *β-Lg* variant A showed a higher frequency over variant B in Holstein cattle (Heidari *et al.*, 2009; Doosti *et al.*, 2011). Badola *et al.* (2003) demonstrated a positive association between AA genotype of *β-Lg* and higher milk yield in Sahiwal cattle and, BB genotype and higher milk yield in Holstein Friesian cattle. The milk produced by BB genotype cows yielded significantly more cheese than that produced by AA genotype cows (Patel *et al.*, 2007). On the other hand, Badola *et al.* (2004) observed no significant effect between milk composition (fat percentage) and *β-Lg* genetic polymorphism.

The $x^2$ test revealed that the calculated value for *β-Lg* gene (1.4525) was lesser than the tabulated value at 5% and 1% level of significance with 2 degrees of freedom. Hence the population under study was found to be in Hardy-Weinberg Equilibrium with respect to *β-Lg* gene. Similar findings were reported also by Rachagani *et al.* (2006) in Sahiwal and Tharparkar cattle breeds, Heidari *et al.* (2009) in Holstein cattle, Karimi *et al.* (2009) in Iranian Najdi cattle, Doosti *et al.* (2011) in Holstein and Iranian native cattle, Gouda *et al.* (2011) in Egyptian Holstein and, Oner *et al.* (2011) in Holstein and Brown Swiss cattle reared in Bursa region.

From the present study it could be concluded that the *β-Lg* B variant was predominant in the indigenous cattle with the highest frequency of BB homozygote followed by AB heterozygote (BB>AB>AA).

## REFERENCES

Aleandri, R., Butazzoni, L.G., Schneider, J.C., Caroli, A. and Davali, R. (1990). The effects of milk protein polymorphisms on milk components and cheese-producing ability. *J. Dairy Sci.*, **73** : 241-255.

Badola, S., Bhattacharya, T. K., Biswas, T. K., Kumar, P. and Sharma, A. (2003). Association of *β*-Lactoglobulin polymorphism with milk production traits in cattle. *Asian-Aust. J. Anim. Sci*., **16** (11) : 1560-1564.

Badola, S., Bhattacharya, T. K., Biswas, T. K., Shivakumar, B. M., Kumar, P. and Sharma, A. (2004). A comparison on polymorphism of Beta-Lactoglobulin gene in *Bos indicus, Bos Taurus* and *Indicine X Taurine* crossbred cattle. *Asian-Aust. J. Anim. Sci*., **17** (6) : 733-736.

Bhattacharya, T.K. and Gandhi, R.S. (1997). Marker assisted selection (MAS) and its application in dairy cattle. *Indian Dairyman.*, **49** : 39-45.

Chianese, L., Di Luccia, A., Mauriello, R., Ferrara, L., Zehender, G. and Addeo, F. (1988). Polimorfismo biochimico delle proteine del latte in bovine di razza Podolica. *Zoot. Nutr. Anim.*, **14** : 189-197.

Del Lama, S.N. and Zago, M.A. (1996). Identification of the k-Casein and *β*- Lactoglobulin genotypes in Brazilian *Bos indicus* and *Bubalus bubalis* populations. *Brazilian J. Genet*., **19** (1) : 73-77.

Doosti, A., Arshi, A., Yaraghi, M. and Dayani-Nia, M. (2011b). Comparative study of *β*-Lactoglobulin gene polymorphism in Holstein and Iranian native cattle. *J. Cell and Anim. Biol*., **5** (3) : 53-55.

Eggen, A. and Fries, R. (1995). An integrated cytogenic and meiotic map of the bovine genome. *Anim Genet*., **26 :** 216–236. Erhardt, G. (1996). Detection of a new ê-Casein variant in the milk of Pinzgauer cattle. *Anim. Gen*., **27** : 105–107.

Gouda, E.M., Galal, M.K., Wasfy, M.A. and Abdelaziz, S.A. (2011). Phenotypes, genotypes and allele frequencies of Beta-Lactoglobulin in Egyptian cattle and buffalo. *J. Agril. Sci.*, **3** (4) : 203-210.

Grosclaude, F. (1988). Le polymorphisme génétique des principales lactoprotéines bovines.Relations avec la quantité, la composition et les aptitudes fromagères du lait. *INRA Prod. Anim.*, **1** (1) : 5-17.

Heidari, M., Azari, M.A., Hasani, S., Khanahmadi, A. and Zerehdaran, S. (2009) Association of genetic variants of *β*-Lactoglobulin gene with milk production in a herd and a superior family of Holstein cattle. *Iranian J. Biotechnol*., **7** (4) : 254-257.

Karimi, K., Nasiri, M.T.B., Fayyazi, J., Mirzadeh, K.H., Roushanfekr, H. (2009). Allele and genotype frequencies of *β*-Lactoglobulin gene in Iranian Najdi cattle and buffalo populations using PCR-RFLP. *African J. Biotechnol*., **8** (15) : 3654-3657.

Lum, L.S., Dovc, P. and Medrano, J.F. (1997). Polymorphisms of Bovine *β-Lactoglobulin* promoter and differences in the binding affinity of Activator Protein-2 Transcription Factor. *J. Dairy Sci*., **80** : 1389-1397.

Marziali, A.S. and Ng-Kwai-Hang, K.F. (1986). Effects of milk composition and genetic polymorphism on cheese composition. *J. Dairy Sci.*, **69** : 2533-2542.

Medrano, J.F. and Aguilar-Cordova, E. (1990). Polymerase chain reaction amplification of bovine â-Lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. *Anim. Biotechnol*., **1** (1) : 73-77.

Moody, D.E., Pomp, D., Newman, S. and MacNeil, M.D. (1996). Characterization of DNA polymorphisms in three populations of Hereford cattle and their associations with growth and maternal EPD in line 1 Herefords. *J. Anim. Sci.* **74** : 1784-1793.

Oner, Y., Pullu, M., Akin, O. and Elmaci, C. (2011). Investigation of *β*-Lactoglobulin (*β-Lg*) and Bovine Growth Hormone (*bGH*) gene polymorphisms by using HaeIII and MspI restriction enzymes in Brown Swiss and Holstein breeds reared in Bursa region. *Kafkas Univ Vet Fak Derg*., **17** (3) : 371-376.

Patel, R.K., Chauhan, J.B., Singh, K.M. and Soni, K.J. (2007). Allelic frequency of Kappa-Casein and Beta-Lactoglobulin in Indian crossbred (*Bos taurus* X *Bos indicus*) dairy bulls. *Turk. J. Vet. Anim. Sci*., **31** (6) : 399-402.

Rachagani, S., Gupta, I.D., Gupta, N. and Gupta, S.C. (2006). Genotyping of â-Lactoglobulin gene by PCR-RFLP in Sahiwal and Tharparkar cattle breeds. *BMC Genet*. **7** : 31-34.

Remus-Alexandru, V.-B, Georgescu, S.E., Manea, M.A., Dinischiotu, A. and Costache, M. (2010). Analysis of Beta-Lactoglobulin and Kappa-Casein genotypes in cattle.

Ren, D.-X., Miao, S.-Y., Chen, Y.-L., Zou, C.-X., Liang, X.-W. and Liu, J.-X. (2011). Genotyping of the ê-Casein and â-Lactoglobulin genes in Chinese Holstein, Jersey and water buffalo by PCR-RFLP. *J. Genet*., **90** (1) : 1-5.

Sambrook, J. and Russell, D.W. (2001). Molecular Cloning: A laboratory manual, 3rd Edn., Cold Spring Harbor laboratory Press, New York.

Sitkowska, B., Neja, W., Wisniewska, E., Mroczkowski, S. and Sawa, A. (2009). Effect of the polymorphic composite forms of Beta-Lactoglobulin on the milk yield and chemical composition in maximum lactation. *J. Cent. Eur. Agric*., **10** (3) : 251-254.

Soller, M. and Beckmann, J.S. (1983). Restriction fragment length polymorphism in genetic improvement. *Theor. Appl. Genet*., **67** : 25-33.