

## GENETIC POLYMORPHISM IN EXON 3 OF PROLACTIN GENE IN INDIAN MURRAH BUFFALOES

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Prolactin plays an important role in mammary gland development, milk secretion, and expression of milk protein genes. Thus, the gene is a potential quantitative trait locus and genetic marker of production traits in dairy animals. Therefore, present study aimed to screen buffalo herd maintained at National Dairy Research Institute, Karnal for the genetic polymorphism of the *PRL* gene using PCR–RFLP analysis for future use of genetic variants as markers for animal selection. Genomic DNA was isolated from 150 Murrah buffaloes. PCR was set up to amplify exon 3 of prolactin locus using specific primers. PCR-RFLP analysis was carried out by digestion of 156 bp amplicon with *Rsa I* restriction enzyme. The animals included in the study exhibited two genotypes AA and AB with frequencies of 0.93 and 0.07 with allelic frequencies of A and B alleles respectively, indicative of very low polymorphic content. DNA sequencing revealed three variations at three nucleotide position, C5398G and G5433A both in exon 3 region out of which C5398G resulting in amino acid substitution from leucine to valine. Third change A5463G was in intron 3 region. Such a methodology of true genotyping of the locus may further be utilized in developing specific breeding programmes for genetic variants for their future use as genetic markers for animal selection.

**Key Words:** Prolactin gene, PCR-RFLP, Nucleotide Variation, Murrah buffalo.

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In Marker-Assisted Selection of dairy cattle, some genes are proposed as potential candidates associated with dairy

performance traits. Among various candidates, prolactin seems to be promising, because it plays a crucial role in mammary gland development and in the initiation and maintenance of lactation. Allelic variation in the structural or regulatory sequences of the prolactin (*PRL*) gene would be of interest because of the possible direct or indirect effect on milk production.

Prolactin is a polypeptide hormone with multiple functions, secreted mainly by the anterior pituitary gland. Prolactin has over 300 separate biological activities (Bole-Feysot *et al.*, 1998) and plays an important regulatory function in mammals, especially dairy animals like the development of mammary gland, milk secretion, and expression of milk protein genes. Hence, the *PRL* gene is a potential quantitative trait locus and genetic marker of production traits in dairy animals. In view of the role of *PRL* considered above, an association of polymorphic variants of the *PRL* gene with milk yield in cattle is probable. The gene has been mapped on chromosome 23 and consists of 5 exons (Camper *et al.*, 1984) encoding the 199-amino-acid mature protein. Within the bovine *PRL* gene, several polymorphisms have been reported to be associated with milk production traits. One of them, recognized by *Rsa I* endonuclease, has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP (Udina *et al.*, 2001; Dybus, 2002; Alipanah *et al.*, 2007). However, only a few reports are available on genetic polymorphism in buffaloes. Therefore, present study aimed to screen buffalo herd maintained at National Dairy Research Institute, Karnal for the

genetic polymorphism in exon 3 of the prolactin gene using PCR–RFLP analysis.

## MATERIALS AND METHODS

### DNA Extraction

Blood samples were collected in vacutainer (Bacton-Dickinson vacutainer system) containing Sodium EDTA as an anticoagulant from 150 Murrah buffaloes maintained at National Dairy Research Institute, Karnal, India. Genomic DNA was extracted from 10 ml of whole blood using standard phenol-chloroform method. The quality of DNA was checked by running on 0.8 % agarose gel and was quantified by reading absorbance at  $A_{260}/A_{280}$  nm in a UV spectrophotometer.

### Polymerase chain reaction

Specific set of cattle primers: Forward: 5'-CGAGTCCTTATGAGCTTGATTCTT-3' and Reverse: 5'-GCCTTCCAGAAGTCGTTTGTTC-3' was used to amplify exon 3 of prolactin locus (Mitra, 1994). Polymerase chain reaction (PCR) was carried out in 25  $\mu$ l of reaction mixture containing 20 pmole of each primer, 200  $\mu$ M dNTP, 1 unit of *Taq* polymerase, 10XPCR buffer and 50 ng of genomic DNA. PCR was performed under the following conditions: denaturation at 94°C for 1 min, annealing at 59°C for 1 min, elongation at 72°C for 1 min (32 cycles) and final elongation at 72°C for 10 min. The PCR amplification was carried out in a programmable thermal cycler (MJ Research, PTC 200). The PCR products were loaded on 1.5% agarose to confirm the amplification of target region using 100 bp DNA ladder.

### PCR-RFLP using *Rsa* I Restriction Enzyme

The amplicons were digested with 8 units of *Rsa* I restriction endonucleases (Bangalore Genei, India) in a final reaction volume of 25  $\mu$ l containing 20.0  $\mu$ l of PCR product 2.00  $\mu$ l of ddH<sub>2</sub>O, 2.5  $\mu$ l of restriction endonucleases buffer and incubated at 37°C for at least 3 hours. The restriction fragments were electrophoresed in 2.5% agarose gel in TBE buffer along with 100 bp DNA ladder as molecular size marker. Gels were stained

in ethidium bromide before visualizing under UV light. The genotype patterns were scored manually in gel documentation system and the allelic frequencies were calculated.

### Identification of Sequence Variation

Amplicons were DNA sequenced from M/s Chromous Biotech., Bangalore and the edited nucleotide sequences were aligned with reference sequences (NCBI accession number AC\_000180). Sequence data were analyzed by using Chromas (Ver. 1.45, <http://www.technelysium.com.au/chromas.html>). Sequence data from variants of different regions were subjected to multiple alignments (Clustal W) for identifying the SNPs.

## RESULTS AND DISCUSSION

High molecular weight DNA was obtained after isolation from in Murrah buffalo blood with overall yield ranging from 350-510  $\mu$ g with a mean of  $414.64 \pm 4.87$   $\mu$ g/ml and the overall purity of DNA ( $OD_{260/280}$ ) ranged from 1.70-1.90 with a mean of  $1.80 \pm 0.01$ . PCR amplification using specific primer and standardized conditions generated 156 bp fragment containing exon 3 of prolactin gene. The amplicons were subjected to restriction digestion using *Rsa* I endonuclease to determine the genotypes in buffaloes. Frequency distribution of *PRL* alleles originated due to a silent A-G transition in the 103 codon (exon 3), which causes the appearance of polymorphic *Rsa*I site. The restriction fragments obtained for the *PRL-Rsa*I polymorphism were: single band of 156 bp (no restriction site) corresponded to genotype AA; three bands of 156 bp, 82 bp and 74 bp fragments corresponded to genotype AB (Figure 1) with respective frequencies of 0.927 and 0.073. Hence, the genotyping procedure revealed three patterns of fragments of 156 bp (allele A) and 82 and 74 bp (allele B). The frequencies of A and B alleles were 0.964 and 0.036 respectively as mentioned in table 1. Genotype BB was not found. Similar results have been reported by Mitra (1994) in Murrah and Nili-Ravi breeds of buffaloes with frequencies of AA and AB genotypes as 0.93; 0.84 and 0.07; 0.16

Table 1: Genotype and allele frequency by *Rsa I* –RFLP of prolactin locus in Murrah buffalo

<i>PRL-Rsa I</i> genotype	Fragment Size (bp)	No. of animals	Genotypic frequency	Allelic frequency	
				A	B
AA	156	139	0.927	0.964	0.036
AB	156, 84, 72	011	0.073		
BB	-	-	0.000		

Table 2: Nucleotide variation in exon 3 of prolactin gene of Murrah buffalo

Nucleotide Position	Region	Change in Base	Type	Amino Acid substitution	Position of Amino Acid
5398	Exon 3	C→G	Non-synonymous	Leu to Val	23 in Exon 3 63 in mature protein
5433	Exon 3	G→A	Synonymous	No Change	---
5463	Intron 3	A→G	---	---	---

Figure 1: Restriction fragments and genotypes of *Rsa I*-RFLP of exon 3 in Murrah buffaloes

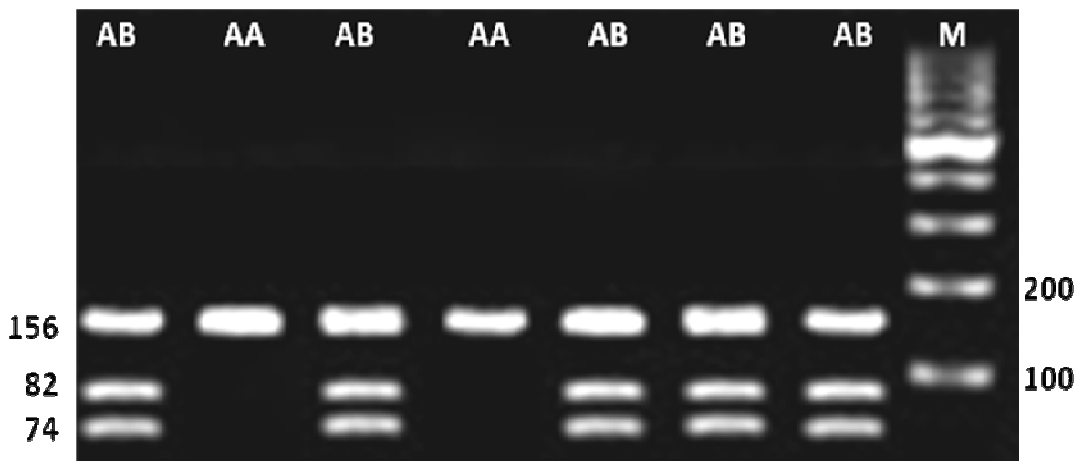


Figure 2: ClustalW alignment of nucleotide sequence and amino acids of exon 3 of prolactin gene in Murrah buffaloes

NUCLEOTIDE SEQUENCE VARIATION

Ref

CCATACCTCCTCCCTTCCTACCCCTGAAGATAAAGAACAAGCCCAACA**G**ACCCACGT  
GAG 5444

query

CCATACCTCCTCC**G**TTTCCTACCCCTGAAGATAAAGAACAAGCCCAACA**A**ACCCACGT  
GAG 137

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Ref

TCTTTTATCCGGGTTTTCACCAGAACAAGTGAGACAGTGCCTGGTGTCCACCAGGCT  
TCA 5504

query TCTTTTATCCGGGTTTTCG----- 156

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AMINO ACID SUBSTITUTION (Leucine to Valine at position 23 of exon 3)

ref

DLSSEMFNEFDKRYAQGKGFITMALNSCHTSSLPTPEDKEQAQQTHHEVLMSLI  
LGLLRS 120

query -----DKRYAQGKGFITMALNSCHTSSVPTPEDKEQAQQTH-----

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respectively. Results are also similar to some of Indian cattle breeds (*Bos indicus*) reported by Kumari et al., (2006). However, Ladani et al. (2003) identified AB and BB genotypes with respective frequencies of 0.965, 0.035; 0.87, 0.13 and 1.00; 0.00 in Surti, Jaffarabadi and Mehsani buffaloes, genotype AA was not identified in any of the breed studied, The frequencies of A allele in Mehsani, Surti and Jaffarabadi were 0.50, 0.48 and 0.43 respectively. Arvindakshan (1997) reported the frequency of A allele to be 0.98 for Surti breed, while Kumar (2004) observed only AA genotype in three breeds of buffalo viz. Mehsana, Jaffrabadi and Surti with frequency of A allele as 1.00 in the three breeds of buffaloes. Tabar et al. (2010) have reported only AA genotype indicating monomorphism in exon 3 of prolactin locus in buffalo population of Khuzestan-Iran. Clustal W alignment of the nucleotide sequences resulted in three variations viz. C5398G- a transition; G5433A- a tranversion, both in exon 3 region. Mutation at 5398 nucleotide position was non-synonymous resulting in amino acid substitution from leucine to valine at position 23 of exon 3 corresponding to position 63 of 199 amino acids long mature protein of prolactin gene (Figure 2), while second mutation was of synonymous type resulting in no change in amino acid. Third change A5463G was in intron 3 region (Table 2). Halabian et al. (2008) have reported four SNPs in *Bos taurus*, three of which were non-synonymous. This observation regarding sequence variation is first report in buffaloes, therefore, results of

any other study in buffaloes were not available for comparison.

## CONCLUSION

The PCR amplification using bovine prolactin gene primer generated 156 bp segment from buffalo prolactin gene homologous to bovine PRL gene. In Murrah buffaloes AA and AB genotypes with respective frequencies of 0.927 and 0.073 were identified indicating more prevalence of AB genotype in the herd. The frequency of allele A and B were 0.964 and 0.036. It may be concluded that the chosen cattle primers were adequate to amplify the sequence of prolactin in *Bubalus bubalis* but the lack of polymorphism is due to species difference. Present study reveals the effectiveness of PCR-RFLP method in differentiating AA and AB prolactin gene variants. In present study, a total of three nucleotide changes have been observed. Two of these were in exon 3, one of which was non-synonymous i.e. has resulted in amino acid substitution from leucine to valine. Third SNP was in intron 3 region. Such a methodology of true genotyping of the locus may further be utilized in developing specific breeding programmes for genetic variants for their future use as genetic markers for animal selection.

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