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Genotyping of β-lactoglobulin Gene in Pakistani Dairy Cattle (Sahiwal Breed) by Using PCR-RFLP

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Abstract
Genetic improvement of livestock is inevitable to increase per animal productivity and meet the dietary requirements of ever growing population. To improve efficiency and economic returns in dairy farming, conventional breeding takes many years to achieve targeted production. However advances in molecular biology have made it possible to identify candidate genes influencing the milk quality as well as yield. As choice of animals for attractive genotypes has been the premise in livestock development, the current research explains the use of Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) technique to categorize bovine Beta-lactoglobulin (β-LG) gene polymorphisms in Sahiwal breed of Pakistani cattle. A segment of β-LG gene exon IV specifically (252 bp), comprised polymorphic positions for A and B allele was effectively amplified by PCR and subsequently digested with *Hae* III restriction enzyme. The yield was two types of restriction pattern i.e. 144 bp, 108 bp, 74 bp and 70 bp (four fragments) for genotype AB and similarly for genotype BB it was 108 bp, 74 bp and 70 bp(three fragments). The observed genotypes were AB and BB with a frequency of 0.60 and 0.40 respectively. Furthermore, frequency of the B allele was found higher (60%) than allele A (40%) in Sahiwal breed. Therefore β-LG genotyping by PCR-RFLP can be used in choosing superior animals for milk traits.
Keywords: Beta-lactoglobulin, Gene polymorphisms, RFLP, Sahiwal cattle.

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INTRODUCTION

Improvement of productivity and financial returns is imperative in dairy farming. The essential objective of dairy industry has been to make out a proficient and economical method to enhance milk generation with no expanding in size in a dairy herd. Milk related genes might be constructive as hereditary indicators used for further choice measure in breeding programs. Polymorphism reported in milk protein genes has evoked significant research consideration in late years as a result of their conceivable affiliations with milk generation as well as composition (Davis *et al.,* 2017). β-Lactoglobulin (β-LG) was the essential milk protein where a polymorphism was recognized through paper electrophoresis and ordered as of β1 and β2 (Rachagani *et al.,* 2006; Archana, 2013).

β-Lactoglobulin gene is located on bovine chromosome 11 and its loci mainly affect the milk quality and production parameters. Up to this point as a minimum15 genetic variants are identified for β-LG and A and B are found to be more successive ones (Zaglool *et al.,* 2016). Every variant encompasses five cysteine deposits from which four are incorporated into confining intra-chain scaffolds of disulphide. The A variant of β-LG contrasts B only by 2 amino acids i.e. aspartate (64) and valine (118), which are replaced with glycine and alanine in B variant, respectively. In bovines especially, allele B is seen as predominant for milk quality while allele A is connected with yield factors (Tsiaras *et al.,* 2005).

Pakistan is fortunate enough in having best dairy *Sahiwal* cattle breed. It is the best milch cattle breed in the tropics and renowned for its higher milk generation, tick-resistant and heat-tolerant abilities. Breeding zones of *Sahiwal* cattle recline in locale of Sahiwal, Okara, Pakpattan, Multan, and Faisalabad districts of Punjab province. This has a wedge and fleshy body form whereas coat shadings vary from reddish dun or sorrel to typical brown (Hassanin, 2014). Male hump is huge as compared to female whereas udder is hefty and muscular along with uniform teats. The general characters for superior milk producers are cows having slack and dropping horns with prominent umbilicus. So realizing the significance of milk production in the economy of Pakistan the existing

examination intends at genotyping the *Sahiwal* cows for β-LG gene.

MATERIALS AND METHODS

Specimen collection

The present study was conducted to evaluate the status of β-Lactoglobulin (β-LG) genetic variants in *Sahiwal* breed animals (n=30) purchased from different districts of Punjab province under veterinarian consultation and maintained at Livestock Research Station (LRS), Animal Sciences Institute (ASI) NARC, Islamabad. 3-5 ml blood samples were obtained aseptically by animal jugular vein in sodium EDTA containing vacutainers and stored at -20ºC until used for DNA extraction.

Genomic DNA extraction

Whole genomic DNA extraction was performed with Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, United States) according to manufacturer's instructions. For transparency and concentration of DNA, all the DNA specimens were analyzed through a Nano Drop (BioSpec-nano Schimadzu Biotech, Life Sciences).

PCR amplification

The primer sequences used here for β-LG gene exon IV amplification (252 bp) were accounted for by Rachagani et al. (2006) with the following nucleotide sequences: 5'-GTC CTT GTG CTG GAC ACC GAC TAC A-3'(F) and 5'-CAG GAC ACC GGC TCC CGG TAT ATG A-3'(R). Reactions were completed in an aggregate volume of 25 µL, contained 2.5 µL 10x buffer, 2.5 µl (2.5 mM) dNTP blend, 1 µL (10 pmol) each of forward and reverse primers, 0.3 µL *Taq* DNA polymerase (5 U/µL), 12.2 µl nuclease free water and 3 µl DNA template (30 ng/µL) in a thermal cycler (Applied Biosystem, USA) with following cyclic conditions: introductory denaturation at 95ºC for 7 minutes took after by 35 cycles for 1 minute at 94ºC, 30 seconds at 60ºC, 45 seconds at 72ºC along a last extension at 72ºC for 15 minutes. All samples were then loaded on 1.2% agarose gel along with a 50-bp DNA ladder marker and estimated through a gel documentation system (Alpha Innotech, California).

RFLP Analysis

For genotyping, specific 252 bp PCR products containing polymorphic positions used for β-LG A and B alleles were digested by Fast Digest *Hae*III restriction enzyme. The reaction was set up in a final volume of 20 µL containing 10 µL reaction solutions, 0.5 µL restriction enzyme, 2 µL enzyme buffer and 7.5 µL ddH_2O and an incubation at 37ºC about 3 hours. All digested products were resolved on 3% agarose gel and envisioned in a gel documentation framework subsequent to ethidium bromide staining.

RESULTS AND DISCUSSION

Since some genes have been proposed as potential candidates for milk performance traits, their molecular investigation is vital for marker assisted selection in dairy breeding programs. From those candidate genes, β-Lactoglobulin influences the quality and production parameters of milk and enhances the estimation of breeding values (Thiruvenkadan *et al.,* 2013). A relationship of β-Lactoglobulin genotypes with milk characteristics has been tended in many bovine breeds recent years (Tahira *et al.,* 2014). In current investigation, electrophoretic investigation of segregated DNA from blood using 0.9% agarose gel demonstrated high molecular bands that indicated that DNA is suitable for PCR-RFLP analysis (Figure 1) and found an OD ratio (260nm/280nm) 1.7 to 1.9. Digestion of all samples were settled on 3% agarose gel and imagined by a gel documentation framework (Alpha Innotech, California). A specific segment of β-Lactoglobulin gene exon IV (252 bp) which involved polymorphic positions for A and B allele was effectively amplified by PCR technique (Figure 2) and restricted with *Hae*III restriction enzyme.

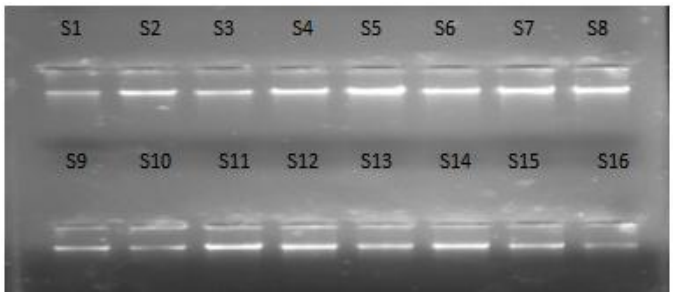

Fig. 1. DNA extraction analysis of *Sahiwal* cattle individuals on a 0.8% agarose gel.

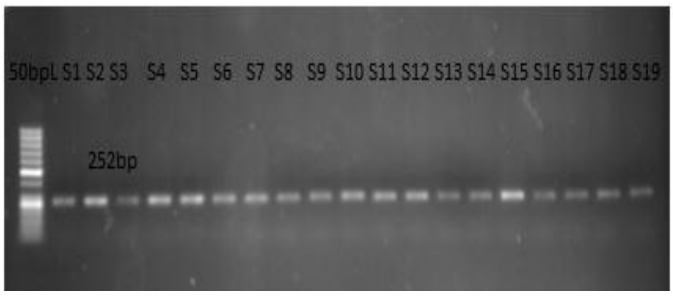

Fig. 2. PCR analysis of β-Lactoglobulin gene exon IV in Sahiwal cattle on a 1.2% agarose gel. Lane 1: 50 bp Ladder, Lane 2 to 19: 252 bp PCR products.

All processed fragments showed two genotypes which are categorized as AB (144 bp, 108 bp, 74 bp and 70 bp) and BB (108 bp, 74 bp and 70 bp) (Figure 3). These results were in accordance with previous study (Rachagani *et al*., 2006). Genotypic recurrence of AB and BB were separately

0.60 and 0.40 in the analyzed Sahiwal cows. Additionally, recurrence of A allele was found lower than B allele and in conformity with the findings of prior workers as Zaglool et al. (2016) who detailed the most noteworthy recurrence of allele B in Holstein-Friesian dairy cattle. Similarly B genotype was the most incessant in indigenous cattle of Assam (Jebin *et al.,* 2016) and in Holstein and Jersey cattle breeds (Ren *et al.,* 2013; Singh *et al.,* 2014). Karimi *et al.* (2009) indicated that milk delivered by β-Lactoglobulin AA genotype dairy animals has been found to hold more lactoglobulin, a lesser amount of casein and fat than that got from BB genotype who yielded significantly more cheese whereas Tsiaras et al. (2005) indicated that β-Lactoglobulin genotype AB is associated with high milk and protein production. Lucak et al. (2013) revealed comparable discoveries for the Serbian Holstein Friesian cows and was similar to that shown by Gouda et al. (2011) in Egyptian Holstein cattle. Hence β-Lactoglobulin genotyping can be utilized in selecting superior individuals for milk production in shorter time than the traditional selection; current genotyping may be useful to increase the frequency of favorable allele or alleles in a dairy breeding program.

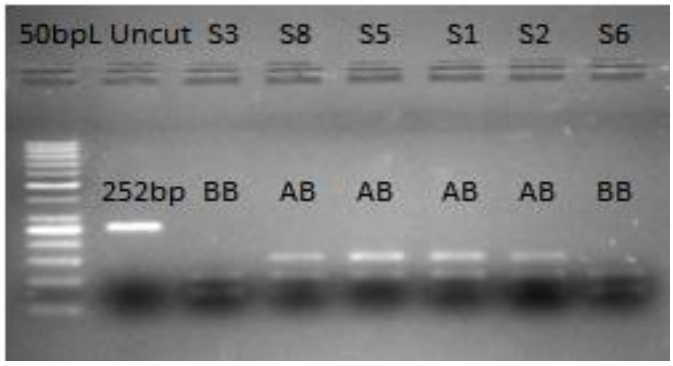

Fig. 3. Restriction investigation of β-Lactoglobulin gene exon IV utilizing *Hea* IIIcatalyst in *Sahiwal* cattle on a 3% agarose gel. Lane 1: 50 bp Ladder, Lane 2: uncut PCR amplicon, Lane 3 and 8: BB genotype and Lane 4 to 7: AB genotype.

CONCLUSION

Henceforth β-LG genotyping by PCR-RFLP can be used in choosing superior animals for milk traits in diminutive time; the data presented here will be useful for improving milk traits by marker assisted selection to increase the frequency of favorable allele or alleles in a dairy breeding program.

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CONFLICT OF INTEREST

The writers have announced that no contending interest exists.

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