Indian Journal of Animal Sciences **90** (4): 655–657, April 2020/Short communication

Growth hormone-Msp1 loci polymorphism and its association with first lactation traits in Sahiwal cattle

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Received: 12 April 2019; Accepted: 22 August 2019

Keywords: Growth hormone, Lactation traits, Msp1, PCR-RFLP, Sahiwal cattle

Sahiwal is considered one of the best indigenous milch breeds of cattle and utilized widely for improvement of indigenous stock in many tropical countries. Various approaches has been tried to increase their productive performance, selection based on molecular biomarkers is one of them. Among different molecular biomarkers, growth hormone (GH) gene is considered one of the most significant genes that can influence lactation traits in dairy animals. Bovine GH (*bGH*) is a single copy gene that spans 1,800 bp, associated with chromosome region 19q26 in bovine genome (Hediger *et al.* 1990), and it consists of five exons separated by introns (Cole *et al.* 2011). Several polymorphisms were identified in the *GH* gene. Leucine or valine amino acid substitutions at residue 127 in *GH* exist due to allelic polymorphism (Sartore and Di Stasio 2000). A polymorphic site for Msp 1 restriction endonuclease was detected by Cowan *et al.* (1989) and Hilbert *et al.* (1989) and the polymorphism being localized in intron 3 of the *GH* gene in position 1,547 (Zhang *et al.* 1993). Studies on the effect of the GH-Msp1 polymorphism on production traits in Sahiwal cattle are quite advanced, but the results obtained by various workers are not always in agreement. The aim of this study was to estimate the allelic frequencies at the GH-Msp 1 loci and to investigate the associations of this polymorphism and first milk production traits in Sahiwal cows.

Sahiwal cows (305) maintained at Livestock Research Centre of ICAR-National Dairy Research Institute (NDRI), Karnal during 1993–2016 were recruited for the present study. Sahiwal cows with a complete lactation were included the statistical analysis (180 Sahiwal cows with minimum 100 days of lactation length and 500 kg of milk yield). The cows with first lactation were used. Blood samples along with records of first lactation 305 days milk yield (FL305DMY), first lactation total milk yield (FLTMY) and first lactation length (FLL) were collected from 305 lactating Sahiwal cattle. DNA from whole blood samples was isolated as per the standard method with slight modification (John *et al.* 1991). For preparing the working solution of genomic DNA, the stock was diluted to100 ng/µL and then stored at –20°C for utilizing it as DNA template in PCR. Agarose gel electrophoresis and UV spectrophotometer were used to analyze the quality and quantity of DNA. The ratio between OD_{260} and OD_{280} was calculated for each DNA sample and sample with ratio of 1.8 was considered good and used for analysis. The GH-Msp 1 genotypes were analyzed using the PCR-RFLP method. A 328 bp fragment of intron 3′ of *GH* gene was amplified by PCR using primer F: 5′-CCCACGGGCAAGAATGAGGC-3′ and R: 5′-TGAGG-AACTGCAGGGGCCCA-3′ (Mittra *et al.* 1995). The PCR protocol to amplify that fragment was denaturation at 95°C for 5 min for 1 cycle, denaturation at 94°C for 30 sec, primer annealing at 68.5°C for 30 sec, PCR products synthesis at 72°C for 30 sec and final synthesis at 72°C for 10 min for 40 cycles. Amplified DNA was digested by Msp1 enzyme at 37°C for 12 h. Reaction mixture consisted of 10 µl PCR product, 2 µl buffer, 0.3 µl (1U) Msp1 and 7.7 µl ddH_2O. The digestion products were electrophorated by horizontal electrophoresis (90 volts, 50 min) using 2% agarose gel in 1×TBE and 1.0 µM ethidium bromide to identify polymorphism of alleles based on the length of the band.

Direct counting of the bands appearing in the gel was used for determination of genotypic frequency in the population. Data for 305 day milk production in first lactation including FL305DMY, FLTMY and FLL were obtained from the record room of the Animal Genetics and Breeding Division of ICAR-NDRI, Karnal. Statistical calculations were performed using SAS procedures. The effect of GH genotypes on the first milk production traits of cows were analyzed using the GLM procedure. The significant effect of SNP variants on first production traits in Sahiwal cows was analyzed using the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where Y_{ij}, the lactation trait of j^{th} animal belonging to i^{th} genotype; μ, the overall population mean; G_i, the effect of i^{th} genotype and e_{ij}, the random error associated with Y_{ij} observation and assumed to be normally and independently distributed with mean zero and constant variance, i.e. NID $(0,\sigma^2_e)$. The genotypic frequencies were calculated using various RFLP patterns. The allelic frequencies were

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*Corresponding author email: shwetagb24@gmail.com calculated using genotypic frequencies. The allelic frequencies were calculated by observing the presence or absence of restriction sites at different alleles.

The 329 bp fragments from the *GH-Msp1* gene was characterized and successfully amplified from the DNA of each sample; this indicated a strong conservation of the DNA sequence existing in cattle. The numbers of Sahiwal cow polymorphism GH locus bearing CC, CT and TT genotype were 4, 54 and 247 respectively (Table 1). The GH TT genotype was found most frequent in the Sahiwal cattle, followed by the CT. The least frequent was the CC genotype. The C genotype was replaced by T genotype at 73rd position of nucleotide (Fig. 1).

Table 1. Genotypic and allelic frequency of different genotype

Locus		Genotype		Frequency	Allele	Frequency
		CC (4)		0.013	C	0.095
Msp1	Intron 3	CT (54)	305	0.177		
		TT (247)		0.809	T	0.898

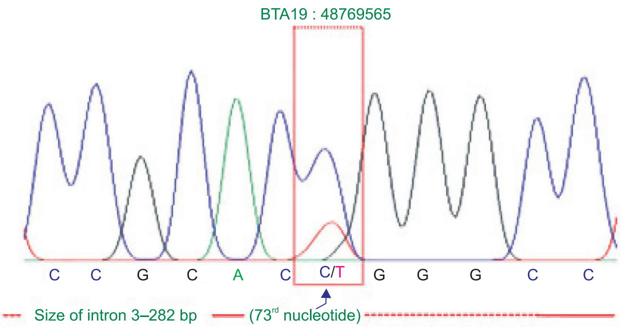

Fig. 1. Chromatogram showing polymorphism at GH-Msp 1 locus.

The frequency distribution of the Msp1 (–) allele was ranging from 0.94 (Sahiwal cattle) to 0.67 (Nagori cattle) and the combined average allelic frequency was 0.87 for Msp1 (–) and 0.13 for Msp1 (+) in Indian cattle breeds (Sodhi *et al.* 2007). Frequencies of GH-Msp1 alleles obtained in this study are in agreement with the findings of Mittra *et al.* (1995) who observed gene frequencies for GH-Msp1 (+) and GH-Msp1 (–) as 0.14 and 0.86 respectively, in Sahiwal cattle. Accordingly, Cerit *et al.* (2014) reported frequencies of 0.107 and 0.893 for GH-Msp1 (+) and GH-Msp1 (-) alleles respectively in Sahiwal cattle. The highest frequency 1 of the GH in Holstein cow was observed by Langziel *et al.* (2000). Higher GH-Msp1 (+) frequency than calculated (0.90 vs. 0.91) was also found by Vukasinovic *et al.* (1999) while Yao *et al.* (1996) reported some what smaller values (0.86 vs. 0.83). The frequency of TT genotype of GH-Msp1 was 1 and same as T allele frequency (Putra *et al.* 2013). Oner *et al.* (2018) observed that the calculated frequency of GH-Msp1 was between 0.40–0.87 in native cattle breeds and predominant allele was A for GH-Msp1 locus. The findings of the present study revealed in Sahiwal cows that there was high variability in the GH locus and offer an opportunity to use GH genotype in marker assisted selection programmes.

Table 2. Association of GH-Msp1 polymorphism with first lactation traits

Locus	Genotype	First lactation trait		
		FL305DMY	FLTMY	FLL
GH -Msp1	TT (42)	2090.12^{a}± 136.22	2383.72^{a}± 175.43	321.83^{a}± 17.45
	TC (29)	1566.31^{b}± 163.93	1756.61^{b}± 211.12	297.51^{ab}± 21.01
	CC (109)	1442.67^{bc}± 84.55	1609.44^{bc}± 108.86	272.23^{b}± 10.83

Means with different superscript in a row differ significantly ($P<0.05$).

Significant differences ($P<0.05$) between cows of different GH-Msp1 genotypes were found in first lactation traits (Table 2). The TT cows had higher FL305DMY, FLTMY and FLL than those with CT and CC genotypes hinting about superiority of TT genotype to other two genotypes for first lactation traits.

GH is an anabolic hormone synthesized and secreted by the somatotroph cells; it plays an important role in lactation, nutrients metabolism, tissue growth and fertility in cows (Lucy 2008, Thidar *et al.* 2008). To date, few studies have investigated the effect of GH-Msp1 polymorphism on lactation traits. Results of the present study showed a significant ($P<0.05$) association between GH-Msp1 polymorphisms and the first lactation traits in Sahiwal cows. In first lactation, the cows with TT genotype had higher milk yield. Similar results were obtained by Yao *et al.* (1996) who showed that GH-Msp1 (+) allele increased milk yield by 300 kg/lactation. Chrenek *et al.* (2003) also reported that GH L/L genotypes were significantly associated with better milk production traits. Other previous studies have also suggested an additive effect of the L allele on milk production and cows with L/L genotypes yield more milk, fat and protein than LV and VV cows (Dybus 2002). Andrzej (2002) reported that Msp1 (+/+) genotypes yielded more milk than other genotypes. This study revealed that superior animals (TT genotype) differ genetically from inferior animals (TC and CC genotypes) mainly in their regulation of nutrient utilization and the GH release (Rejduch 2008).

SUMMARY

GH has important role in milk production and their polymorphisms may alter quality and quantity of gene's products and affects the phenotype. This study was designed to find the polymorphisms on GH-Msp1 loci and to find its association with milk production traits in Sahiwal cattle. Msp1 polymorphism of the *bGH* gene was identified in a population of Sahiwal cows. PCR-RFLP genotyping of 329 bp fragment of GH in exon 3 revealed three different allelic variants. The T allele was the major allele in Sahiwal cattle. The TT genotype Sahiwal cows yielded more FL305DMY, FLTMY and also had higher FLL than that of CT and CC genotypes cows. Results of the present findings reveal that

TT allele of GH locus should be the favoured genotype in Sahiwal cattle breed and can be used in marker-assisted selection programmes.

ACKNOWLEDGEMENTS

The first author acknowledges Indian Council of Agriculture Research (ICAR), New Delhi, India and National Dairy Research Institute (NDRI), Karnal, India for financial assistance to carry out this research work. The authors also gratefully acknowledge assistance of working staff of Animal Genetics and Breeding Division and Livestock Research Farm, NDRI, Karnal.

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