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Genetic Polymorphism Study in Prolactin Receptor (*PRLR*) Gene and their Association with Milk Production Traits in Indian Cattle Breeds

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ABSTRACT

The aim of the current study was to investigate the status of Prolactin receptor (*PRLR*) gene polymorphism using PCR-RFLP assay and their association with milk production traits in Sahiwal (n=53) and Hariana (n=50) cattle. Two region of *PRLR* gene consisting of exon 10 and exon 9 revealed 168 and 582 bp products, respectively. PRLR/*Sml*I assay resulted in three types of genotypes, namely, GG (168 bp), TT (123 and 45 bp) and heterozygous GT (168, 123 and 45 bp) genotypes with frequencies 27.18, 6.60 and 67.90%, respectively. The allelic frequency of G and T alleles were 0.607 and 0.393, respectively. The *Dra*III/ PCR-RFLP assay revealed three types of genotypes, namely, AA (582 bp), GG (399 and 183 bp) and heterozygous AG (582, 399 and 183 bp) genotypes with frequencies 17.0, 16.0 and 67.0 %, respectively. The allelic frequency of A and G alleles were 0.505 and 0.495, respectively. Chi square analysis revealed that screened cattle population was found in Hardy-Weinberg equilibrium for both the SNP. Association studies of PRLR/*Sml*I genotypes had significant difference for total milk yield (TMY) and milk yield in 300 days (MY300) among three genotypes. The GG genotype showed higher milk yield value than TT and GT animals in first lactation. PRLR/*Dra*III genotypes had no association with any production traits in first and second lactation. Therefore, the present study demonstrating that G allele of *PRLR/Sml*I gene could be used as a strong marker for improvement in milk production performance in milch cattle.

Keywords: Prolactin receptor, Hariana, Sahiwal, *Sml*I, *Dra*III, polymorphism.

Prolactin receptor (*PRLR*) is a strong candidate gene play important role in the milk production and reproduction. In cattle, prolactin is major specific hormones and their action mediated by *PRLR* genes (Bole-Feysot *et al.,* 1998; Auchtung *et al.,* 2005; Lee *et al.,* 2007, Beauchemin *et al.,* 2006, Curi *et al.,* 2006). The bovine *PRLR* is mapped on chromosome 20 comprises of three exons and two introns. The *PRLR* has been detected in various tissues including ovary, placenta and uterus in several mammalian species (Shirota *et al.,* 1990). Mice homozygous for null mutations in *PRLR* are sterile due to a failure of embryonic implantation, demonstrate irregular cycles, reduced fertilization rates and defective embryonic development (Ormandy *et al.,* 2002).

Sahiwal and Hariana breeds of cattle have star performer status as the premier dairy animal of the India with high potential for milk, draught power and farm manure production. Sahiwal is best milch and Hariana is best dual purpose breed in India (Nivsarkar, 2000). Hence, there is needs to conservation of Sahiwal and Hariana along with selection for the desirable production and reproduction traits, because to improve the production potential to cope up with the ever increasing demand of milk and milk products for constantly increasing human population of India. Polymorphism study of *PRLR* gene and its effects on milk production traits explore the possibilities of *PRLR* gene being used as candidate marker gene for milk production traits.

Several single nucleotide polymorphisms (SNPs) studies in *PRLR* gene have been reported in different cattle breeds including Jersey and Polish Black and white cattle (Brym *et al.,* 2005), Finish Ayreshire cattle (Vitala *et al.,* 2006), Chinese Holstein cattle (Zhang *et al.,* 2008). However, there is only one report (Deepika and Salar, 2014) available in Indian cattle breeds including Hariana but they did not analyze any association with milk production and reproduction traits. Keeping this in view, the present study was undertaken to detect the *PRLR* polymorphism using PCR-RFLP assay and its association with milk production and reproduction traits in Indian Hariana and Sahiwal cattle breeds.

MATERIALS AND METHODS

Animals and DNA isolation

A total of 103 adult female of Sahiwal (n = 53) and Hariana (n = 50) cattle breed were utilized in the present investigation, maintained at Instructional Livestock Farm Complex, DUVASU, Mathura (Uttar Pradesh). The animals were kept in identical environmental conditions and were fed a standard diet on the farms. Incomplete lactations for any recorded reason or ending with abortion or other anomaly were deleted.

Approximately 5 ml blood from jugular vein of cattle was collected in 10 ml of vacutainer tubes containing EDTA as anticoagulant. Genomic DNA was isolated using the standard phenol/chloroform extraction method (Sambrook and Russel, 1991). The concentration and purity of genomic DNA was determined spectrophotometrically at OD_{260} and OD_{280}. The integrity of the DNA was examined by agarose gel (0.7%) electrophoresis and the gel was visualized under UV light after staining with ethidium bromide (EtBr).

Amplification of *PRLR* gene fragments and PCR-RFLP assay

Two SNPs (*G>T* and *A>G*) containing regions of *PRLR* gene fragments have been amplified from isolated DNA using primer pairs described as per Deepika and Salar (2014) and Javed *et al.* (2011), respectively (Table 1). The PCR amplification was carried out in a total volume of 25 µl that contained 1.0 µl of genomic DNA (50-100 nmoles), 1 X PCR buffer (10 mM Tris–HCl, pH 8.8 at 25 ^{o}C, 50 mM KCl), 2.5 mM of $MgCl_2$, 2.5 mM of each dNTPs (Fermentas, USA), 5 pmoles of each primer and one unit of *Taq* DNA polymerase (Fermentas, USA). The restriction digestion was carried out at 37^{o}C for 14-16 hrs in a total volume of 15 µl containing 10 µl PCR products, 1.0 µl (10 U/µl) and 1.5 µl 10X RE buffer. For restriction fragment analysis, digested products were checked on 2.0% agarose gel in 1X TAE buffer for 4-5 hrs at 5 V/cm. The fragments were visualized under UV light after staining with EtBr.

Statistical analysis

The data was generated by estimating the frequency of different digested products. The allelic and genotypic frequencies of different *PRLR* genotypes were estimated by standard procedure (Falconer and Mackay, 1996). The chi square (χ^2) test ($P \leq 0.05$) was also performed to test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium (Snedecor and Cochran, 1989).

Association study

The association study of *PRLR* genotypes with the following milk production and reproduction traits: Lactation period (LP = date of drying – date of calving), Total milk yield (TMY = calculated by totaling of daily milk records of individual cow after completion of their lactation.), Milk yield in 300 days (MY300 = calculated by totaling of daily milk records of individual cows up to 300 days of lactation), Dry period (DP = date of calving – date of drying), Calving interval (CI = difference between two successive calving), Peak yield (PY= obtained by recording the maximum amount of milk given by an individual cow for the first time since onset of lactation), Days to reach peak yield (DRPY = date of peak yield – date of calving) was performed. Statistical analysis of milk production and reproduction traits in relation to *PRLR* genotypes was carried out using the General Linear Model (GLM) using SPSS software. The following linear model was applied:

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

Where Y_{ij}– observed trait value in animal; µ – mean

trait value; G_i – effect of genotype; e_{ij}– random error. Significant differences among least square means of different genotypes were calculated using Duncan's multiple-range test, and P values of 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The amplified fragments of the *G>T* SNP containing *PRLR* exon 10 region revealed about 168 bp PCR product (Fig. 1).

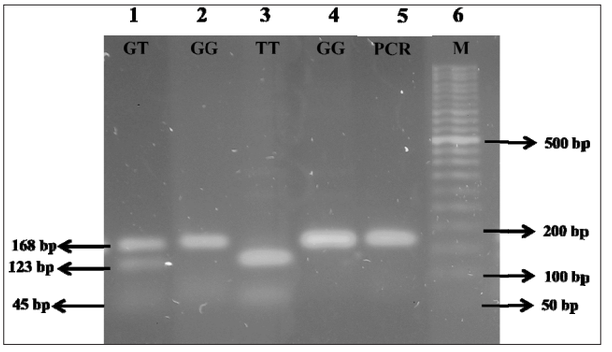

Fig. 1: PRLR/*Sml*I PCR-RFLP assay showing genotype pattern in 2.0% agarose gel; Lanes 1: GT genotype (168, 123 and 45 bp); Lane 2, 4: GG genotype (168 bp only); Lanes 3: TT genotype (123 and 45 bp); Lane 5: Undigested PCR product (168 bp), M=Marker (50 bp)

The *PRLR*/*Sml*I PCR-RFLP assay revealed three types of banding pattern (genotypes); one of them was 168 bp (GG genotype); second of 168, 123 and 45 bp (GT genotype) and third of 123 and 45 bp (TT genotype) (Fig. 1). This revealed that the screened cattle population used in the present study was polymorphic in nature with two types of alleles G and T. Genotypic and allelic frequencies of *PRLR*/*Sml*I genotypes are presented in Table 2. Chi square test revealed that $\chi^2_{cal\,(0.959)} < \chi^2_{tab\,(5.99)}$ at 5% level of significance for degree of freedom 1 indicating that screened cattle population was in Hardy-Weinberg equilibrium (Table 2).

PRLR/*Sml*I genotypic and allelic frequencies in present study and in different cattle breeds as observed by other authors are presented in Table 3. The value of GG genotype (28.0%) in Hariana breed in the present study was not in accordance with the findings of Deepika and Salar (2014) in Hariana cattle (41.30%). However, the total frequency of GG genotype (27.18%) in screened cattle population, was in the range (21.7% to 56.3%) observed in other indigenous grey cattle (Deepika and Salar, 2014). This variation in frequency of GG genotype might be due to population size and geographical distribution. The genotypic frequency of GT genotype in Hariana and Sahiwal cattle was 64.0% and 69.81%, respectively, which was in agreement with the observations of Deepika and Salar (2014) in various breeds of Indian cattle ranges

Table 1: SNPs, Primers, annealing temperature and restriction enzyme details of amplified regions of *PRLR* gene

SNP	Region	Primer sequence	Product Size	Ann Temp	RE
G>T	Exon 10	5'- AGATGGAGTGCTGGCTCTGT-3' 5'- GCCTTCTTGGCTGGTTCTTC-3'	168 bp	60°c	*Sml*I
A>G	Exon 9	5'-CAACATTGCTGACGTTGTGTG-3' 5'- CAATTGAACCCATCCTTCCA-3'	582 bp	63°c	*Dra*III

Table 2: Allelic and genotypic frequencies of *G>T* SNP/*Sml*I gene in cattle breeds

Breed		Genotypic frequency (%)			Allelic Frequency	
		GG	GT	TT	G	T
Sahiwal (N=53)		26.41 (n=14)	69.81 (n=36)	3.78 (n=03)	0.613	0.387
Hariana (N=50)		28.00 (n=14)	64.00 (n=32)	8.00 (n=04)	0.60	0.40
Total (N=103)		**27.18 (n=28)**	**67.90 (n=68)**	**6.6** (n=07)	0.607	0.393
Number	Expected	36.84	23.85	15.44	χ2 cal = 0.9598 χ2 tab = 5.991 (*P<0.05*) χ2 cal < χ2 tab	

Table 3: Genotypic and allelic frequencies of *PRLR*/*Sml*I gene in different cattle breeds

Breed	Genotypic frequency			Allelic frequency		Reference
	GG (%)	GT (%)	TT (%)	G	T	
❑ Hariana	41.30	52.20	6.50	0.674	0.326	Deepika and Salar (2014)
❑ Kankrej	38.60	61.40	0.00	0.693	0.307	
❑ Mewati	55.80	32.60	11.60	0.721	0.279	
❑ Nagori	21.70	45.70	32.60	0.446	0.554	
❑ Tharparkar	39.60	56.30	4.20	0.677	0.323	
❑ Ghumusari	48.90	36.20	14.90	0.670	0.330	
❑ Hill cattle	56.30	41.70	2.10	0.771	0.229	
❑ Kangayam	37.50	29.20	33.30	0.521	0.479	
❑ Binjharpuri	52.10	37.50	10.40	0.708	0.292	
❑ Punganur	36.10	38.90	25.00	0.556	0.444	
❑ Hariana	26.41	69.81	3.78	0.613	0.387	In present study
❑ Sahiwal	28.00	64.00	8.00	0.60	0.40	In present study

Table 4: Association studies of *PRLR*/*Sml*I genotypes with milk production and reproduction traits in Indian cattle breeds

Lact	Breed	Geno	n	LP	TMY	MY300	DP	CI	PY	DRPY
I	Sahiwal (N=41)	GG	13	360.8±28.2	2150.9±172.7	1769.4±95.5^{b}	321.0±36.0	533.3±46.6	7.15±0.5	62.0±9.6
		GT	25	350.0±21.4	1901.1±152.0	1551.9±85.6^{ab}	259.2±23.6	507.9±29.4	7.14±0.4	64.2±7.7
		TT	3	384.6±55.8	1633.5±287.1	1264.0±42.6^{a}	309.3±79.6	541.0±41.6	7.83±1.4	52.0±20.9
	Hariana (N=40)	GG	11	365.9±41.2	1519.9±279.9	1205.7±206.9	330.5±63.3	472.3±23.7	5.6±0.5	43.8±6.0
		GT	25	362.5±17.9	1600.8±128.4	1312.7±87.26	269.6±36.7	537.0±23.8	6.2±0.4	46.2±3.0
		TT	4	290.0±67.3	1102.0±202.2	1052.2±231.0	332.0±127.2	452.7±45.8	4.8±1.0	49.5±13.0
	Total (N=81)	GG	24	363.1±23.1	1861.7±168.8^{b}	1511.0±120.8^{b}	325.4±34.1	504.1±27.0	6.4±0.5	53.7±6.0
		GT	50	356.2±13.9	1751.2±101.5^{b}	1432.6±62.8^{b}	263.0±21.8	523.2±18.4	6.6±0.3	55.8±4.3
		TT	7	330.5±46.7	1329.2±137.2^{a}	1143.6±101.9^{a}	322.5±71.9	490.0±34.8	6.1±1.0	50.3±10.5
II	Sahiwal (N=28)	GG	10	348.2±56.0	1957.0±366.3	1368.6±218.9	322.7±54.5	430.0±62.0	7.1±1.1	45.9±13.6
		GT	16	309.3±32.2	1773.9±211.9	1470.6±153.6	251.6±27.7	444.6±18.9	8.03±0.6	51.1±6.5
		TT	2	408.0±123.0	2345.2±116.3	1809.5±419.5	266.0±3.0	481.5±136.5	8.75±2.7	20.5±2.5
	Hariana (N=38)	GG	11	294.6±29.2	1427.8±179.5	1314.9±148.7	218.2±40.3	495.4±33.8	6.9±0.8	55.5±6.7
		GT	23	339.7±24.9	1605.7±142.7	1348.8±107.23	223.6±33.8	488.0±16.4	6.9±0.5	49.8±4.3
		TT	4	224.7±19.2	1009.5±227.1	1009.5±227.1	363.5±120.0	392.5±46.5	5.6±1.2	42.7±7.5
	Total (N=66)	GG	21	320.1±30.5	1679.8±201.7	1340.5±126.8	270.4±35.1	467.8±32.5	6.9±0.6	50.9±7.2
		GT	39	327.2±19.6	1674.7±120.0	1398.8±88.5	235.0±22.8	470.2±12.7	7.3±0.4	50.3±3.6
		TT	6	285.8±51.5	1454.8±354.5	1276.2±246.6	331.0±78.9	422.2±49.6	6.6±1.2	35.3±6.7

Different letters in superscript of a given row indicates significant ($P<0.05$) difference between genotypes, n — number of individuals in particular genotype, N — total number of individual in particular breed, Lact=Lactation; Geno = Genotype

36.2 to 61.4%. The heterozygote frequency being higher than homozygotes showed it would be effective to select for or against polymorphism in this population of animals. The value of TT genotype of Hariana and Sahiwal cattle was 8.0% and 3.78%, respectively, which was on lower side of the range (0.0% to 32.3%) observed by Deepika and Salar (2014) in different breeds of Indian cattle. It may be due to different native tract of indigenous cattle breeds. In present study, the G allele of *PRLR*/*Sml*I gene in total screened cattle population was 0.607, which was higher

than T allele frequency (0.393). It was in accordance to the findings (G=0.645 and T=0.355) of Deepika and Salar (2014) in different Indian breeds of cattle (Table 3).

The means and standard errors of mean (Mean ± S.E.M.) of each studied trait related to *PRLR*/*Sml*I genotype in first and second lactations are presented in Table 4. There was significant difference observed for the TMY among all the genotypes in the first (*P=0.042*) lactation of screened cattle. There was also significant (*P=0.045*) difference for the MY300 among all three genotypes in first lactation. The animals with GG and GT genotypes have more milk yield than TT genotype. The MY300 for Sahiwal cattle with GG genotype was also higher than TT genotype. No significant difference was observed for LP, DP, CI, PY and DRPY among all the *PRLR*/*Sml*I genotypes in both lactations (Table 4). Association between *PRLR* variants and milk production traits have been reported by various authors (Scotti *et al.,* 2003; Vitala *et al.,* 2006; Zhang *et al*., 2008 and Lu *et al*., 2011). Scotti *et al*. (2003) analyzed two *PRLR* polymorphisms in the Reggiana cattle breed and association with milk, protein and fat yield. Brym *et al.* (2005) reported one SNP (A→C) in *PRLR* intron 9 for Jersey and Polish Black-and-White cattle and observed association with protein content in milk. Variation in *PRLR* is reported to be significantly associated with milk yield in Finnish Ayrshire dairy cattle (Vitala *et al.,* 2006). Zhang *et al.* (2008) detected polymorphisms in *PRLR* gene using PCR-SSCP in Chinese Holstein dairy cattle and observed association between *PRLR* variants and milk performance traits. Lu *et al.* (2011) detected the SNPs within exon 10 of the *PRLR* gene in Chinese cattle breeds using PCR-SSCP and their association with growth traits.

The amplified fragments of the *A>G* SNP containing *PRLR* exon 9 region revealed about 582 bp product by performing 1.5% agarose gel electrophoresis (Fig. 2).

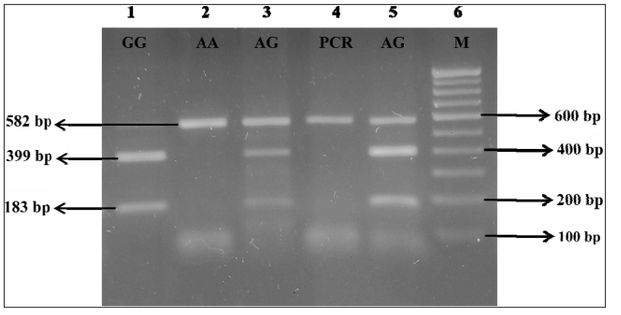

Fig. 2: PRLR/*Dra*III PCR-RFLP assay showing genotype pattern in 1.5% agarose gel; Lanes 1: GG genotype (399 and 183 bp); Lane 2: AA genotype (582 bp only); Lanes 3, 5: AG genotype (582, 399 and 183 bp); Lane 4: Undigested PCR product (582 bp), M=Marker (100 bp)

The *PRLR*/*Dra*III PCR-RFLP assay revealed three types of banding pattern (genotypes); one of them was 582 bp (AA genotype); second of 582, 399 and 183 bp (AG genotype) and third of 399 and 183 bp (GG genotype) (Fig. 2). It revealed that the screened animals used in the present study were polymorphic in nature with two types of alleles A and G. Genotypic and allelic frequencies of *PRLR*/*Dra*III genotypes are presented in Table 5. Chi square test revealed that $\chi^2_{cal\ (1.43)} < \chi^2_{tab\ (5.99)}$ at 5% level of significance for degree of freedom 1 indicating that screened cattle population was in Hardy-Weinberg equilibrium (Table 5).

The means and standard errors of mean (Mean ± S.E.M.) of each studied trait related to *PRLR*/*Dra*III genotype in first and second lactations are presented in Table 5. There was no significant difference observed for the LP, TMY, MY300, DP, CI, PY and DRPY among all the *PRLR*/*Dra*III genotypes in all the lactations (Table 6). However, there was no report of *PRLR*/*Dra*III polymorphism in any cattle breeds. Javed *et al.* (2011) investigated this SNP in Indian buffalo as AA (65%), AG (30.0%) and GG (3.9%). This

Table 5: Genotypic and allelic frequencies of *A>G* SNP/*Dra*III genotypes in Indian cattle breeds

Breed		Genotypic frequency (%)			Allelic Frequency	
		AA	AG	GG	A	G
Sahiwal (N=50)		16.0 (n=8)	72.0 (n=36)	12.0 (n=6)	0.52	0.48
Hariana (N=50)		18.0 (n=9)	62.00 (n=31)	20.00 (n=10)	0.49	0.51
Total (N=100)		**17.0 (n=17)**	**67.00 (n=67)**	**16.0 (n=16)**	**0.505**	**0.495**
Number	**Expected**	25.50	49.99	24.50	χ2 cal = 1.43 χ2 tab = 5.991 (*P<0.05*) χ2 cal < χ2 tab	

Table 6: Association studies of *PRLR*/*Dra*III *genotypes* with milk production and reproduction traits in Indian cattle breeds

Lact	Breed	Geno	n	LP	TMY	MY300	DP	CI	PY	DRPY
I	**Sahiwal (N=40)**	**AA**	4	371.8±53.9	1914.3±234.6	1538.2±197.8	311.5±545.1	533.3±46.6	7.5±4.9	59.7±21.1
		AG	29	336.2±22.5	1934.5±159.3	1598.5±104.7	277.2±230.6	502.4±28.9	7.0±6.25	66±50.4
		GG	7	399.3±29.3	1919.5±267.6	1452.9±210.2	290.8±168.5	579.3±42.7	8.8±.04	66.2±50.2
	Hariana (N=40)	AA	6	403±55.2	1997±428.5	1597±2.5	336.8±236.8	336.5±236.8	5.83±2.5	47.5±22.8
		AG	25	337.5±18.0	1409.9±30.6	1189.2±474.7	279.2±81.6	279.2±181.6	5.6±1.9	43.1±15.9
		GG	9	376.5±43.8	1546.3±234.4	1219.4±389.1	300.5±221	300.5±221.5	6.67±2	52.5±18.7
	Total (N=80)	**AA**	10	390.2±120.3	1963.2±828.3	1573±599.2	326.1±196.5	512.3±117.8	6.5±2.2	52.4±22.3
		AG	54	336.6±107.5	1691.3±789.4	1409.2±559.3	278±151	503.1±111.8	6.4±2.2	54.1±29.9
		GG	16	386.5±108.2	1709.2±707.9	1321.2±767.3	296.1±181.3	536.3±168.2	6.7±1.9	58.6±33.5
II	**Sahiwal (N=27)**	**AA**	3	226.3±46.1	872.0±672.0	823.3±631.7	244.3±104.4	385±**10**9.3	4.27±2.7	48±49.4
		AG	20	341.3±144.1	2026.7±903.0	1568±613.3	255.9±128.8	446.2±99.3	8.2±2.6	51.1±6.5
		GG	4	342.5±184.5	1816.8±1052	1305±575	357.5±196.3	416.3±188.2	7.5±2.8	48.55±36.1
	Hariana (N=38)	**AA**	6	324.8±118.2	1427.5±623.2	1260±456	292.6±224.3	465.2±83.6	6±2.5	37.8±11.3
		AG	24	320.5±120.7	1526.7±708.1	1302.5±535.2	238.1±258.6	485.1±102.1	6.7±2.3	53.7±21.5
		GG	8	289.7±86.5	1143.5±559.0	1338.1±502.4	191.3±138	475.3±81.5	7.1±2.8	51.1±21.2
	Total (N = 65)	**AA**	9	292.2±128.3	1242.3±657.2	1114.4±426.2	276.3±186.5	454.2±82.2	5.5±2.5	41.2±26.5
		AG	44	329.3±130.1	1754.2±834.6	1423.5±580.3	246.2±143.3	470.2±101.6	7.4±2.5	51.3±28.5
		GG	12	307.3±121.4	1561.2±732.2	1327.1±487.2	246.7±171.7	455.9±121.3	7.2±2.5	58.1±21.8

Different letters in superscript of a given row indicates significant ($P<0.05$) difference between genotypes, n — number of individuals in particular genotype, N — total number of individual in particular breed, Lact = Lactation; Geno = Genotype

is our first report of *PRLR*/*Dra*III polymorphisms (*A>G* SNP) and their association with milk production traits in Indian cattle breeds. In the present study, we reported that the screened cattle were found polymorphic for *A>G* SNP in *PRLR* exon 9 region. The allelic frequency of allele A and G was calculated as 0.52 & 0.48 in Sahiwal and 0.49 & 0.51 in Hariana breed, which was different from 0.816 & 0.184 found in Indian buffalo, respectively (Javed *et al.,* 2011). Javed *et al.* (2011) investigated this polymorphism in Indian buffalo but association study was not done with milk production traits. The current study was the first report of association with milk production traits but no association was found with milk production traits in Indian cattle breeds.

CONCLUSION

The current study was the first report of *PRLR* polymorphism and their association with milk production traits in Indian cattle breeds including Sahiwal and Hariana. In case of *G>T* SNP in *PRLR* exon 10 investigation, the animal with GG genotype was found significantly associated with higher milk yield than TT genotype. So it was concluded that G allele is responsible for higher milk yield than T allele in studied cattle breeds. While *A>G* SNP in *PRLR* exon 9 had no association with milk production traits in present study. Therefore, this information about *PRLR* gene could be useful in marker assisted selection to improve the milk production performance in dairy cattle.

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