



Association of *PIT-1* gene polymorphism with milk production traits in Sahiwal cattle

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ABSTRACT

In the present study, identification of *PIT-1* gene polymorphism and its association with milk production traits was undertaken in 77 Sahiwal cattle maintained at NDRI, Karnal using PCR-RFLP. Amplification of DNA sample revealed 600 bp product and restriction digestion with *HinfI* showed three types of genotypes, namely, AA (600 bp), AB (600, 343 and 257 bp) and BB (343 and 257 bp) genotypes. The frequency of BB genotypes was highest (64.98%) in all screened samples, followed by AB genotype (31.16%) and AA genotype (3.89%). The allelic frequency of *PIT-1* A and B alleles were 19.48% and 80.51%, respectively. Association studies of *PIT-1* gene with milk production traits showed that age at first calving, gestation period and lactation period had non-significant variation among all the 3 genotypes up to fourth lactation. However, a significant difference was found among the 3 genotypes for total milk yield and milk yield at 300 days with AA genotype showing higher value than AB and BB genotypes in the first lactation. From present investigation, the *HinfI* /PCR-RFLP revealed polymorphic pattern of *PIT-1* gene in Sahiwal cattle and association studies showed significant effect of A allele on milk production.

Key words: Milk production traits, PCR-RFLP, *PIT-1*, Polymorphism, Sahiwal

PIT-1/growth hormone factor-1 (*GHF-1*) or *POU1F1* is a pituitary-specific transcription factor that is responsible for pituitary development and hormones expression in mammals (Cohen *et al.* 1997). Various naturally occurring single nucleotide polymorphisms (SNPs) have been reported in *PIT-1* gene and associated with body weight, average daily gains and milk production traits in cattle (Renaville *et al.* 1997, Parmentier *et al.* 1999). The polymorphism in *PIT-1* gene has been widely studied in exotic cattle (Selvaggi and Dario 2011, Ozdemir 2012) but only few studies done in Indian breeds of cattle including Sahiwal breed (Mukesh *et al.* 2008). Considering limited study in Sahiwal cattle, this study was undertaken to investigate the status of *PIT-1* gene polymorphisms and their association with milk production traits in Sahiwal cattle breeds.

MATERIALS AND METHODS

Animals' source, DNA Extraction and *HinfI*/PCR-RFLP: A total of 77 females of Sahiwal cattle maintained at

Instructional Dairy Farm, National Dairy Research Institute (NDRI), Karnal (Haryana), were utilized in the present investigation. Genomic DNA was extracted from venous blood using the standard protocol of Rao *et al.* (1994). The primers used for amplification of *PIT-1* gene ('F': 5'-GAG CCT ACA TGA GAC AAG CAT C-3' and 'R': 5'-AAA TGT ACA ATG TGC CTT CTG A-3') were as per Javanmard *et al.* (2010). The restriction digestion was carried out at 37°C for 14 h in a total volume of 15 µl containing 5.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 units of 1.0 µl *HinfI* enzyme.

Statistical analysis: The data was generated by estimating the frequency of different RFLP pattern (genotypes). The allelic frequency and genotypic frequencies of *PIT-1* gene was estimated by standard procedure (Falconer and Mackay 1996).

Association study: The association study of *PIT-1* genotypes with milk production traits (Age at first calving; AFC, gestation period; GP, dry period; DP, lactation period; LP, total milk yield; TMY, milk yield at 300 days; MY 300 days) was performed and analyzed by one way ANOVA with the help of SAS (version 9.2).

RESULTS AND DISCUSSION

The amplified product of *PIT-1* gene was 600bp (Fig.1). The *HinfI*/PCR-RFLP assay and sequence analysis revealed three types of genotypes; one of them was of 600 bp (AA genotype); second of 343 and 257 bp (BB genotype); third

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Table 1. Genotypic and allelic frequencies of *PIT-1* gene in Sahiwal cattle

Breed	Genotypic frequency (%)			Allelic frequency (%)	
	AA	AB	BB	A	B
Sahiwal (N=77)	3.89 (n=3)	31.16 (n=24)	64.93 (n=50)	19.48	80.51

of 600, 343 and 257 bp (AB genotype) (Fig.1). The allelic and genotypic frequencies were calculated and presented in Table 1. In the present study on Sahiwal breed of cattle, the value of AA genotypic frequency (3.89) was not in accordance with the findings of Mukesh *et al.* (2008) in Sahiwal cattle (0.0). However, it was in agreement with the results of Mukesh *et al.* (2008) in Dangi cattle (2.0), Nassiri *et al.* (2010) in Najdi cattle (3.57) and Ozdemir (2012) in Holstein cows (4.0). The genotypic frequency of

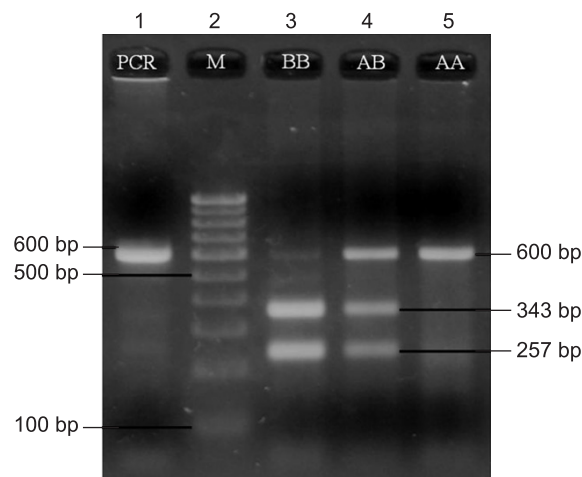


Fig. 1. *PIT-1*/Hinf I PCR-RFLP assay showing genotype pattern in 2.0% agarose gel; Lane 1: Undigested PCR product, 2: Marker (100 bp ladder), 3: BB genotype (343 & 257 bp), 4: AB genotype (600, 343 & 257 bp), 5: AA genotype (600 bp only).

Table 2. Association studies of *PIT-1* genotypes with milk production traits

Lactation	Genotype	n	Age at first calving (days)	Gestation period (days)	Dry period (days)	Lactation period (days)	Total milk yield (liter)	Milk yield 300 days (liter)
I(N=77)	AA	3	1048.00±33.06	283.33±2.66	99.00±48.50	337.67±19.09	2085.3±433.03 ^b	1913.7±364.64 ^b
	AB	24	1191.90±45.86	283.33±4.90	134.33±16.75	327.42±18.73	1558.9±122.48 ^a	1280.5±98.94 ^a
	BB	50	1227.00±41.03	283.72±2.31	150.64±14.66	324.24±13.08	1416.3±50.44 ^a	1249.0±43.45 ^a
II(N=71)	AA	3	—	292.00±6.65	126.00±24.00	333.67±67.30	2045.3±663.66	1759.7±418.07
	AB	24	—	284.67±3.23	153.50±23.87	286.92±19.01	1436.9±123.52	1263.1±103.25
	BB	44	—	283.59±1.63	161.91±17.55	303.00±17.99	1496.6±102.70	1275.0±82.93
III(N=63)	AA	3	—	284.67±3.84	85.00±40.95	316.33±53.32	1917.0±537.20	1773.0±460.25
	AB	20	—	286.40±1.64	137.40±26.07	315.80±23.58	1687.7±79.93	1465.3±66.61
	BB	40	—	283.00±1.69	163.80±17.68	289.15±13.10	1513.6±91.53	1377.2±76.98
IV(N=57)	AA	3	—	285.33±1.85	46.66±25.09 ^a	295.33±34.83	1665.7±412.46	1531.7±288.30
	AB	18	—	257.44±11.24	64.44±15.32 ^a	340.78±13.90	1504.4±74.53	1335.2±65.49
	BB	36	—	286.28±3.88	140.11±16.81 ^b	334.50±10.66	1705.1±78.35	1503.5±65.48

'b' indicate significant difference than 'a'.

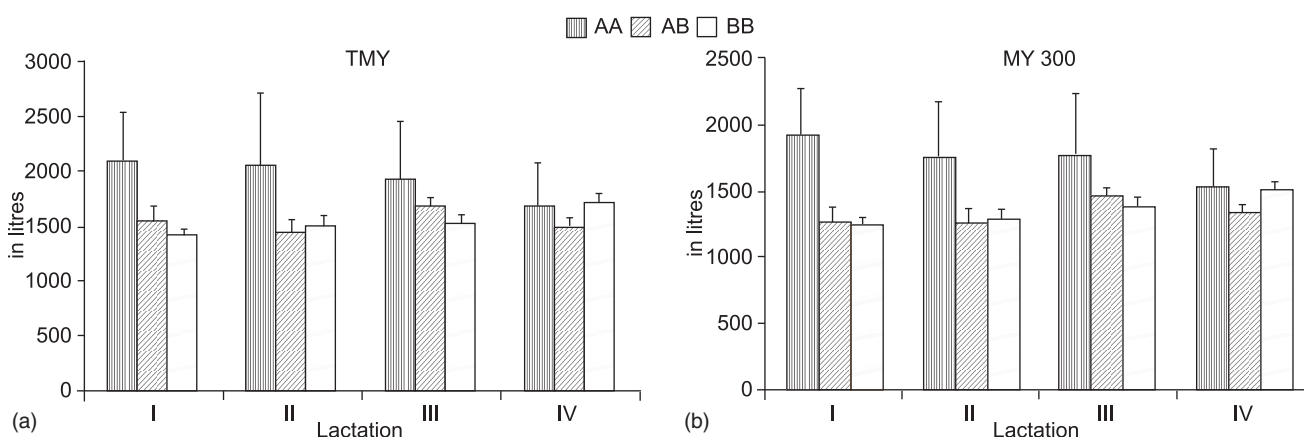


Fig. 2. Graphical representation of association study of *PIT-1*/Hinf I genotypes with (a) total milk yield (b) milk yield at 300 days.

AB genotype (31.16) in the present investigation was in agreement with the observations of Mukesh *et al.* (2008) in Sahiwal (25.9), Doosti *et al.* (2011) in Iranian native cattle (37.5), Selvaggi and Dario (2011) in Podolica cattle (31.73)

and Ozdemir (2012) in Holstein cows (31.0). The frequency of BB genotype (64.93) obtained in present study was consistent with the result of Mukesh *et al.* (2008) in Sahiwal cattle (74.1) and Nassiri *et al.* (2010) in Najdi cattle (66.66)

and Ozdemir (2012) in Holstein cows (65.0) but was higher than the observed values of Doosti *et al.* (2011), Salvaggi and Dario (2011) in various cattle breeds.

Contrary to the present findings in Sahiwal cattle breed, higher frequency of PIT-1/*Hinf*I A allele was reported in some Iranian cattle, viz. *Bos taurus* (68.5) and *Bos indicus* (84.5) (Javanmard *et al.* 2010), Iranian native cattle (74.99) (Doosti *et al.* 2011) and Native Ear Anatolian Red (EAR) cattle (74.99) (Ozdemir 2012). The frequency of PIT-1/*Hinf*I B allele was higher than A allele in the present study. This was reciprocated by the findings of various workers except those of Javanmard *et al.* (2010) in *Bos taurus* and in *Bos indicus*.

The overall mean $\pm$ SE of AFC, GP, DP, LP, TMY, MY 300 days in first, second, third and fourth lactations are given in Table 2. Association studies of *PIT-1* gene with milk production traits showed that AFC, GP and LP had non-significant variation among all the three genotypes upto fourth lactation. However, significant difference ($P \leq 0.05$) was observed for the DP in the fourth lactation (Table 2). A significant difference ($P = 0.038$) was also found among the 3 genotypes for TMY and MY 300 days in the first lactation. AA genotype had higher TMY and MY 300 days than the AB and BB genotypes (Fig. 2). A significantly higher milk yield by individuals of AA genotypes indicated that allele A of *PIT-1* gene might be responsible for higher milk production. Similar finding was also observed by Renaville *et al.* (1997), Parmentier *et al.* (1999), Oshima and Barreras (2003) and Doosti *et al.* (2011) in different cattle breeds.

In the present study, we report the *PIT-1* gene polymorphism in Sahiwal cattle and its association with milk production traits. *Hinf*I/PCR-RFLP assay indicated AA, AB and BB genotypes. Association studies showed that genotype had significant effect on total milk yield and milk yield at 300 days and A allele had significant effect on milk production and can be utilized as a genetic marker.

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