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PIT 1 gene polymorphism and seasonality affect milk production traits in dairy cattle of Kashmir

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ABSTRACT

The study aimed at identifying the best genotypes for important candidate PIT 1 gene and seasonality influencing the yield and quality of milk, in the Jersey and Crossbred HF cattle population maintained at an organized farm in Mountain Livestock Research Institute, Manasbal, Kashmir. The Jersey and Crossbred HF cows (60 each) were genotyped for quality and yield traits. Milk samples (50 ml) were collected on weekly basis for quality evaluation for the years 2016-18, among all the four seasons of Kashmir. The milk quality traits studied were fat%, lactose%, protein%, SNF, density and ash. The quantity traits were average monthly milk yield, protein yield and fat yield. Genomic DNA was extracted using Phenol-chloroform method and association analysis was done. The PIT 1 exon VI (451 bp) was screened for polymorphisms using PCR-RFLP using *Hinf*I restriction enzyme, revealing two genotypes (Pp and pp) for both the breeds. Allele 'p' was most frequently present in Crossbred HF cows (0.95) and Jersey cows (0.82). The genotype Pp in crossbred HF cows and pp in Jersey cows showed higher values for yield traits in all four seasons under study. In crossbred HF cows, the effect of genotype Pp was significant on fat% and SNF. The genotype pp effect was significant on fat% in Jersey cows. Winter and autumn seasons had significant effect on fat% in both breeds under study. Based on the study, it was concluded that breed, season, and genotype of the PIT 1 gene are highly associated with the milk quality and yield in the dairy cattle of Kashmir. Genome wide association studies are recommended for better understanding the effects of all genes on milk quality and quantity.

Keywords: Milk yield, PIT 1 gene, Polymorphism, Quality, Season

The variation in milk production is a regular phenomenon in all milking animals and the factors responsible for such variations are physiological, which are governed by the genetic makeup and environment, such as age, number of previous lactations, pregnancy, season of calving, calving interval and nutritional status. The season of calving has got a marked effect on the total production (Bernabucci *et al.* 2002). Therefore, to enhance the productivity of dairy animals, it is necessary to develop an understanding of the factors affecting both milk production and composition. Genetic factors like the presence of candidate genes and QTLs have also been carried out to determine their influence on economical traits like milk production, protein content, protein quality and body fat percentages in cattle (Yudin and Voevoda 2015). A number of candidate genes have been reported that affect the milk production traits in cattle, viz. β-lactoglobulin (Medrano and Aguilar 1990, Nazir *et al.* 2009, Shah *et al.* 2021b), FMO3 (Lunden *et al.* 2002), DGAT (Grisart *et al.* 2002), BGRH (Blott *et al.* 2003), Leptin gene (Nkrumah *et al.* 2005), Prolactin gene (Aijun Lu *et al.* 2011, Khaizaran and Al-Razem 2014), Kappa- casein (Batista *et al.* 2015, Khairy *et al.* 2015, Shah *et al.* 2021a), Pituitary transcription factor (Khaizaran and Al-Razem 2014), α-lactalbumin (Zhou and Dong 2013, Mir *et al.* 2014).The PIT 1 is a pituitary specific transcription factor that is responsible for pituitary development and hormone expression in mammals (Cohen *et al.* 1997).

PIT 1 gene is considered to be important in improving the milk production due to its role in activating the expression of prolactin, thyrotropin, and growth hormone genes in the anterior pituitary gland (Tuggle and Trenkle 1996). PIT 1 gene is considered to be responsible for the regulation of growth and development in cattle and other mammals (Zhang *et al.* 2009, Heidari *et al.* 2012). The bovine PIT 1 gene is located in chromosome 1 (BTA1) and midway between TGLA57 and RM95 markers (Moody *et al.* 1995). The PIT 1 gene including five introns and six exons code for a protein consisting of 291 amino acids (~33 kD) with DNA-binding POU domain. A correlation between PIT 1

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exon VI gene polymorphism with production and milk quality traits has often been found in many studies on various breeds showing that allele A of the PIT-1 locus positively affects milk production traits (Pan *et al.* 2008, Selvaggi *et al.* 2011, Aytekin İ and Boztepe 2013, Chauhan *et al.* 2016). HF cows were introduced long back in J&K under crossbreeding improvement plan of native cattle germplasm. Till date no research has been done on these crossbred cows. Thus, information on association of PIT 1 exon VI polymorphism in crossbred HF cows remains rare. PIT 1 gene and its polymorphism has been identified in Kashmir cattle population (Shah *et al.* 2021c). It is therefore important to understand the association of seasons and PIT 1 exon VI gene polymorphism on yield and quality of milk in Jersey and crossbred HF cows under the temperate climatic conditions of Kashmir.

MATERIALS AND METHODS

Sample collection: Milk production records of Jersey and Crossbred HF cows (60 each) maintained at an organized farm of Mountain Livestock Research Institute (MLRI) of SKUAST-K were collected on a weekly basis for the years 2016-18. These records were classified according to seasons, viz. spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). The data was normalized by removal of extreme outliers. Since the season is supposed to levy a profound influence on the milk production trends in the same year, data were collected for all four seasons of Kashmir. Milk samples of 50 ml were collected and analyzed for quality for fat, protein, SNF, lactose, density, and ash parameters using Speedy Lab Milk Auto-analyser. The quantity traits were total milk, protein yield, and fat yield.

Blood samples, DNA extraction, purification and quantification: Blood (10 ml) was collected from the jugular vein of each animal in a 15 ml sterile graduated polypropylene tube containing EDTA (0.5 M, *p*H=8.0). The standard phenol-chloroform extraction method was used for genomic DNA extraction (Sambrook and Russell 2001). The quality and quantity of the DNA were verified using a spectrophotometer by checking absorbance at 260 nm for concentration and 260/280 nm for purity. Working dilution of extracted DNA was prepared for each sample at a concentration of 50 ng/µl.

PCR amplification and RFLP: Primer pair: forward 5′AAACCATCATCTCCCTTCTT3′ and reverse 5′AATGTACAATGTGCCTTCTGAG 3′ by Renaville *et al.* (1997) were used for amplification of Exon VI (451 bp). PCR was carried out as per the protocol given in Table 1. The PCR reaction products were electrophoresed on 1.5% agarose gel. Ethidium bromide was used to detect the amplification success. The PCR products were digested with 5 units of *Hinf*I (Thermo Scientific) at 37°C for 1 h in a final reaction volume of 25 µl. The restricted fragments, after restriction digestion, were analysed electrophoretically in 2% agarose gel, with ethidium bromide. The digested products were visualized under UV light on a transilluminator. The banding patterns were scored manually, and gels were recorded in Gel Documentation System.

Table 1. PCR condition for amplification of PIT 1 gene

Step	Temperature (°C)		Time
Initial denaturation	94		2 min
Cyclic denaturation	94	36 Cycles	30 sec
Annealing	57		30 sec
Cyclic extension	72		45 sec
Final extension	72		10 min

Statistical analysis and association study: The frequency of different genotypes and alleles were calculated by using Popgene 1.31 software (Yeh *et al.* 1999). To analyse the Hardy-Weinberg equilibrium of the population, software Popgene version 1.31 was used (Yeh *et al.* 1999) for estimation of observed and expected genotype frequencies. The following general linear model was used for obtaining the association followed by Tukey's test using SAS 9.3 statistical software.

$$Y_{ijkL} = \mu + B_j + G_k + S_L + E_{ijkL}$$

where, Y_{ijkL}, milk yield/fat yield/ protein yield/ SNF/ density/ash/lactose of i^{th} animal of j^{th} breed with k^{th} genotype and L^{th} season; µ, overall mean; Bj, Jersey and Crossbred HF cows; Gk, genotypes for selected gene; SL, season and E_{ijkL}, random error.

RESULTS AND DISCUSSION

PCR amplification and polymorphism analysis: Both the breeds showed a single specific band of 451 bp of exon VI (Fig. 1). Normally on digestion with *Hinf*I restriction enzyme, three patterns PP (451), Pp (451/244/207) and pp (244/207) are found, but in our study, the pattern of two bands (244/207, pp genotype) and three bands (451/244/207 Pp genotypes) were observed and genotype 'PP' was absent (Fig. 2). Our results are in contrast with the findings of Renaville *et al.* 1997b, Hori- shima and Barreras-Serrano 2003, Zhao *et al.* 2004, Yan *et al.* 2006, Zhang *et al.* 2009, Beigi Nassiri *et al.* 2010, Ozdemir 2012 and Hoseinzadeh *et al.* 2015, who proved the presence of all three genotypes in the different breeds. The presence of only two genotypes might

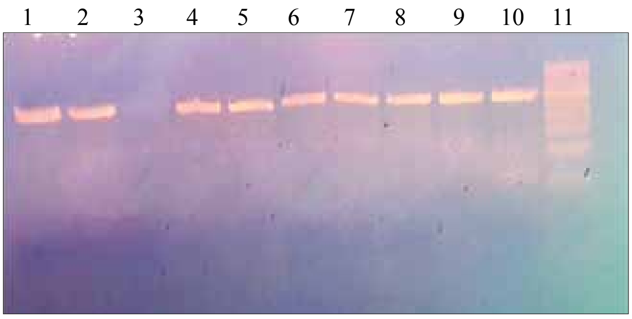

Fig. 1. Amplification of PIT 1 exon VI (451 bp). Note: Lanes 1 2, 4, 5: Jersey cows; Lanes 6-10: Crossbred HF cows; Lane 3: control; Lane 11: 100 bp marker.

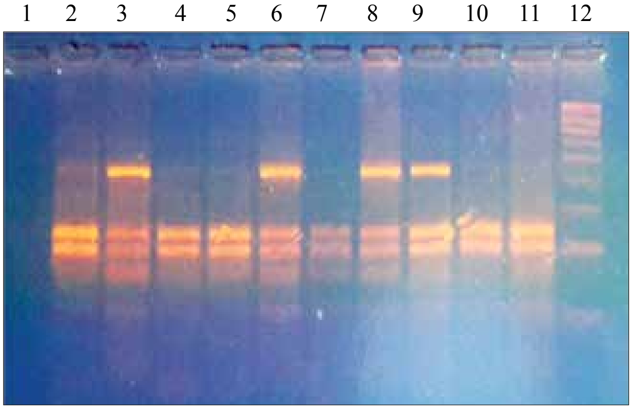

Fig. 2. Amplification of PIT 1 exon VI (451 bp). Note: Lanes 1 2, 4, 5: Jersey cows; Lanes 6-10: Crossbred HF cows; Lane 3: control; Lane 11: 100 bp marker.

be due to a smaller population size or due to selection pressure because MLRI is the only organized cattle farm in Kashmir under temperate conditions which limits the scope for increasing the population size for the study.

Gene and genotype frequencies: The PP genotype was absent within the experimental animals. The heterozygote Pp genotype was most abundant in Jersey cows (0.36) and lowest in crossbred HF cows (0.09). The pp genotype was found highest in crossbred HF cows (0.91). The frequency of the P allele was highest in Jersey cows (0.18). Crossbred HF cows harboured the highest frequency of the p allele (0.95) (Table 2). Similar results were found in double-muscled Blue cattle of Belgia (Renaville *et al.* 1997b), Nanyang cattle (Xue *et al.* 2006), and East-Anatolian Red breed (Ozdemir 2012). When compared with the literature in terms of allele frequencies, our situation was in contrast to the previous studies on Italian Holstein-Friesian, Holstein (Hori-Oshima and Barreras-Serrano 2003), Black-and-White bulls, Angus beef cattle (Zhao *et al.* 2004), Black-and-White cows, Holstein-Friesian (Vargas *et al.* 2004), Chinese Holstein cattle (Yan *et al.* 2006), Holstein cows (Edriss *et al.* 2008) and various crossbred cattle (Zhang *et al.* 2009), Najdi cattle (Beigi Nassiri *et al.* 2010) and Holstein (Ozdemir 2012).

Association studies: Effects of breed, season and genotype were significant ($p<0.05$) for all yield traits under study. The genotype Pp in crossbred cows and pp in Jersey cows showed higher values for MY, PY, and FY in all four seasons under study (Table 3). In Crossbred HF cows the effect of genotype was significant ($p<0.05$) on fat% and SNF and seasons (winter and autumn) were significant ($p<0.05$) for fat% and density (Table 4). The effect of genotype pp was significantly ($p<0.05$) different on fat%, and winter and autumn seasons were found to be effective ($p<0.05$) on fat% (Table 5). Our results are different from Hori and Barreras (2003) who studied the PIT 1 gene polymorphism in Baja California Holstein cattle revealing that the AA genotype for PIT 1 had a significant effect ($p<0.05$) on milk yield as was also reported by Renaville *et al.* (1998) in Italian Holstein-Friesian bulls, Black-and-White bulls, Angus beef cattle (Zhao *et al.* 2004), Black-and-White cows, Holstein-Friesian (Vargas *et al.* 2004), Qinchuan cattle and Chinese Holstein cattle (Yan *et al.* 2006), Manzadrani, Sarabi, Holstein Cows (Edriss *et al.* 2008), crossbreds (Zhang *et al.* 2009), Najdi cattle (Beigi Nassiri *et al.* 2010) and Holstein breed

Table 2. Genotypic and allelic frequencies of PIT1 in Crossbred and Jersey cows

Gene	Genotype	Frequency	Allele	Frequency	Chi-square
PIT 1	Pp	0.09	P	0.05	0.199
Crossbred	pp	0.91	p	0.95	
	Pp	0.18	P	0.18	0.01
Jersey	pp	0.82	p	0.82	p>0.05; NS

Table 3. Effect of PIT 1 genotype, breed and season on milk yield traits

Season	Breed	Genotype	Av. milk yield (MY) (kg)	Av. protein yield (PY) (kg)	Av. fat yield (FY) kg
Spring	Crossbred	Pp	190.63±11.28^{b}	0.73±0.23^{a}	0.79±0.20^{a}
		pp	236.11±11.38^{a}	0.68±0.22^{b}	0.58±1.48^{b}
	Jersey	Pp	160.76±11.21^{b}	0.73±0.49^{b}	0.72±0.03^{b}
		pp	280.45±11.48^{a}	0.89±0.12^{a}	1.02±0.01^{a}
Summer	Crossbred	Pp	300.62±11.48^{a}	1.0±0.16^{a}	0.91±0.06^{a}
		pp	262.36±10.98^{b}	0.77±0.12^{b}	0.68±0.04^{b}
	Jersey	Pp	290.84±15.12^{b}	1.00±0.66^{b}	0.98±0.24^{b}
		pp	345.32±12.01^{a}	1.30±0.82^{a}	1.21±0.26^{a}
Autumn	Crossbred	Pp	262.87±11.48^{a}	0.80±0.16^{a}	1.05±0.11^{a}
		pp	124.92±11.03^{b}	0.68±0.18^{b}	0.89±0.06^{b}
	Jersey	Pp	162.32±12.01^{b}	0.56±0.34^{b}	0.89±0.11^{b}
		pp	285.55±11.03^{a}	0.88±0.23^{a}	1.30±0.48^{a}
Winter	Crossbred	Pp	180.34±16.32^{a}	0.56±0.02^{a}	0.81±0.38^{a}
		pp	120.12±18.16^{b}	0.39±0.12^{b}	0.79±0.23^{b}
	Jersey	Pp	140.36±15.82^{b}	0.53±0.49^{b}	1.01±0.73^{b}
		pp	193.23±17.28^{a}	0.60±0.38^{a}	1.16±0.11^{a}

$^{a,\,b}$Mean with same superscripts are not significantly different ($p<0.05$) from one another; NS, Non-significant.

Table 4. Effect of PIT 1 genotype, breed and season on milk quality traits in Crossbred cows

Parameter	Protein (%)	Lactose (%)	Fat (%)	SNF (%)	Density	Ash (%)
Genotype						
Pp	3.21±0.00^{NS}	4.51±0.02^{NS}	4.48±0.24^{a}	8.32±0.03^{a}	26.62±0.19^{NS}	0.70±0.00^{NS}
pp	3.14±0.01	4.44±0.01	4.18±0.12^{b}	8.65±0.02^{b}	26.96±0.15	0.69±0.01
Season						
Winter	3.17±0.02^{NS}	4.45±0.01^{NS}	4.15±0.11^{a}	8.55±0.02^{NS}	26.61±0.13^{b}	0.69±0.00^{NS}
Spring	3.19±0.01	4.50±0.01	3.01±0.13^{b}	8.61±0.03	26.97±0.15^{a}	0.69±0.00
Summer	3.17±0.02	4.50±0.01	3.51±0.13^{b}	8.55±0.02	26.97±0.15^{a}	0.69±0.00
Autumn	3.19±0.01	4.55±0.01	4.15±0.11^{a}	8.61±0.03	26.61±0.13^{b}	0.69±0.00

NS, Non-significant; ^{a,b,c}Means with same superscripts are not significantly different ($p<0.05$) from one another.

Table 5. Effect of PIT 1 genotype, breed and season on milk quality traits in Jersey cows

Parameter	Protein (%)	Lactose (%)	Fat (%)	SNF (%)	Density	Ash (%)
Genotype						
Pp	3.06±0.02^{NS}	4.34±0.02^{NS}	4.03±0.24^{b}	8.34±0.03^{NS}	26.32±0.19^{NS}	0.68±0.00^{NS}
pp	3.09±0.03	4.36±0.02	5.26±0.25^{a}	8.39±0.02	26.00±0.15	0.68±0.00
Season						
Winter	3.06±0.01^{NS}	4.34±0.01^{NS}	5.27±0.11^{a}	8.32±0.02^{NS}	26.19±0.20^{NS}	0.68±0.01^{NS}
Spring	3.09±0.02	4.36±0.01	4.02±0.13^{b}	8.41±0.00	26.13±0.24	0.68±0.01
Summer	3.06±0.01	4.34±0.01	4.02±0.13^{b}	8.32±0.02	26.19±0.20	0.68±0.01
Autumn	3.09±0.02	4.36±0.01	5.27±0.11^{a}	8.41±0.00	26.13±0.24	0.68±0.01

NS, Non-significant; ^{a,b,c}Means with same superscripts are not significantly different ($p<0.05$) from one another.

(Ozdemir 2012). Salgado Pardo *et al.* (2022) also reported a positive association with milk production traits.

The populations of Crossbred HF and Jersey cattle were polymorphic and p allele was commonly found. Based on the results, it is concluded that breed, season and genotype of PIT 1 gene are highly associated with milk quality and yield in the dairy cattle of Kashmir.

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