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Identification of genetic variants of *bGH*, *GHR* and *IGF1* gene and their association with lactation persistency in Tharparkar cattle

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ABSTRACT

Persistency can be explained as number of days during which the high level of milk production is maintained after the peak milk yield. The aim of this study was to identify genetic variants of bovine-Growth Hormone (bGH), Growth Hormone Receptor (GHR) and Insulin like Growth Factor1 (IGF1) gene and their association with lactation persistency in Tharparkar cattle. Study was carried out using data of daily milk yield, monthly test days, total milk yield and peak yield of 372 lactation records of 190 Tharparkar cattle maintained at Livestock Research Centre of ICAR-National Dairy Research Institute from 1996-2019. Lactation persistency was estimated using Woods and Wilminks method and its least squares means were 2.37±0.08 and -5.4±0.56, respectively. Investigations on polymorphism were performed using seven reported primers for amplifying targeted regions of *bGH*, *GHR* and *IGF1* gene. In PCR-RFLP, *bGH-Msp*I RFLP had shown polymorphism in intron 3 and its genotypic frequencies were TT 0.49, CT 0.39 and CC 0.12. The allelic frequency of C and T were 0.31 and 0.69, respectively. The *bGH-Alu*I RFLP had shown monomorphism in exon 5. GHR-*Alu*I had shown polymorphism in exon 10 with only two genotypes AA and AG with frequency of 0.92 and 0.08, respectively. The frequency of A and G allele was 0.96 and 0.04, respectively. In *GHR-Stu*I and *IGF1-Tas*I in promoter region, *RsaI* in exon 3 and *SnaB* I in exon 1 had shown monomorphism. No significant association was found between genetic variants of *bGH* and *GHR* with lactation persistency in Tharparkar cattle.

Keywords: Growth hormone, *IGF1*, PCR-RFLP, Tharparkar, Woods and Wilminks method

India has become self-sufficient in milk production yet the gap between achieved and achievable milk production is wide. According to the livestock statistics, average daily milk production of indigenous cattle is very poor (3.2 kg in 2020-2021) compared to that of crossbred and exotic cattle (8.2 kg in 2020-2021) (www.data.gov.in). Low productivity might be due to poor genetic potential of indigenous cattle coupled with numerous environmental factors such as lower plane of nutrition and improper management systems (Kabi *et al*. 2016). Tharparkar, a well-known breed of dairy cattle with better heat tolerance, disease resistance and succeed under harsh climatic conditions. In view of the importance of this breed, efforts need to be made for their maintenance and for its genetic improvement. One of the important criteria for productivity of any lactating cow is maintenance of peak yield for a longer period. Persistency is the ability to continue a constant yield throughout the lactation (Gengler 1996). High degree of persistency during the first lactation is desirable and taken as one of the selection strategies for dairy animals (Strabel *et al.* 2001).

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The bovine growth hormone (bGH) has great influence on the lactating bovine mammary gland by controlling the proliferative and apoptotic status of cells and preservation of mammary cell population. The bGH controls the growth and metabolism of cells by growth hormone receptors (GHR) which is present on the surface of desirable cells. Insulin-like growth factor I mediates the growth promoting and metabolic actions of the somatotropin. Cell proliferation and apoptosis is regulated by the receptors and the binding proteins of IGF1.

For more precise and efficient selection in breeding programmes, genetic markers are being used. The *bGH*, *GHR* and *IGF1* gene variants have been taken as specific markers for lactation persistency analysis because of its previously reported significant association with milk production traits. The objective of the study was to identify the factors affecting persistency and; to find the possible association of *bGH*, *GHR* and *IGF1* gene polymorphism with persistency of lactation in Tharparkar cattle in order to identify the potential markers for selection programmes in improvement of Tharparkar cattle.

MATERIALS AND METHODS

A total of 372 milk yield records (305-day/less) of all the lactations at daily milk yield basis from 1996 to 2019

(20 years) were collected for 190 Tharparkar cows sired by 38 bulls from history sheets and daily milk records maintained at Animal Genetics and Breeding Division of NDRI, Karnal. Blood samples were collected from the same 190 lactating Tharparkar cattle and Phenol-chloroform method, as described by Sambrook and Russsel (2001) with minor modifications, was used for DNA isolation.

Each year was classified into four seasons namely winter (December-March), summer (April-June), rainy (July-September) and autumn (October-November) based on agro-climatic conditions and period of calving was classified into six groups (1996-1999), (2000-2003), (2004-2007), (2008-2011), (2012-2015), (2016-2019) to estimate the effect of non-genetic factor on milk production. Lactation persistency was estimated using mathematical method of lactation curve like, woods (incomplete gamma function) model (Woods 1967) and Wilmink function model (Wilmink 1987). Difference among least squares means was tested by using DMRT modified by Kramer (1957). The correlation of persistency of lactation with peak yield, lactation length and total milk production was estimated by standard correlation formula.

Polymerase chain reaction (PCR) amplification of targeted region of bGH, GHR and IGF1 gene and Restriction fragment length polymorphism (RFLP) analysis: Previously reported primers were used to amplify each region of bovine *GH*, *GHR* and *IGF1* gene for PCR-RFLP (Supplementary Table 1). For all primers denaturation was done at 95°C for 3 min. The PCR products were digested with restriction enzymes. The restriction digested fragments were separated on 2.5% agarose gels and resolved by ethidium bromide. Photographs were taken using gel documentation system. The recognition sequence, reaction time and temperature of different restriction enzymes is shown in Supplementary Table 2.

Association of bGH, GHR and IGF1 gene variants with lactation persistency: Association of *bGH*, *GHR* and *IGF1* SNP genotypes with persistency of lactation in Tharparkar cattle was analysed using fixed model least squares analysis (Harvey 1990). The significant effect of SNP variants on persistency of lactation in Tharparkar cattle were analysed using the following model.

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

Where, $Y_{i,}$ observation on j^{th} animal having i^{th} genotype; μ, overall mean; $G_{i,}$ fixed effect of i^{th} genotype of SNP, $e_{ij,}$ random error NID $(0, \sigma^2_{e)}$.

RESULTS AND DISCUSSION

The overall least squares mean for Woods (P1) and Wilminks model (P2) were 2.37±0.08 and -5.4±0.56, respectively and the standard error as percentage of mean for persistency estimates were 3.5 and 9.2 percentage, respectively as shown in Table 2.

The average persistency estimate ranged between 6.70 (Wood 1967) to 72 (Tekerli *et al*. 2000) on the basis of 305-day milk yield, while some workers estimated average persistency based on test-day milk yields ranging between -0.39 to 142±3.8 (Shanks *et al.*1981). Singh *et al*. (1997) observed an average persistency of -0.43 using monthly milk yields. However, some workers also used weekly milk yields and reported an average persistency of 75.89±23.7 (Fadlelmoula *et al*. 2007). Indices for estimation of persistency were compared on the basis of standard error (SE) expressed as per cent of population mean. The lower the standard error expressed as per cent of population mean, the higher would be efficiency of the persistency estimates (Kaushal *et al*. 2016). Woods method has lower standard error as per cent of population mean than Wilmink's method in the present study.

Woods incomplete gamma function method (P1): Mean persistency estimated by gamma function was 2.37±0.08 and first lactation had less persistency than overall mean persistency (2.35±0.17) in Tharparkar cattle. Wider range of persistency estimates have also been reported by Wood (1967), Tekerli *et al*. (2000), Rekik *et al*. (2003) and Atashi *et al.* (2006).

Wilminks method (P2): The average persistency of milk yield was estimated as -5.4 ±0.56. In Tharparkar cattle, the overall lactation persistency was different from first lactation persistency and first lactation had shown maximum persistency (-4.55±1.08). The reported first lactation persistency in Sahiwal cattle (Sachan 2020) was lower than the present estimate. In crossbred cattle higher persistency was testified by Yamazaki *et al.* (2014) and Otwinowska *et al*. (2016). The difference in the estimates of average persistency reported by different researchers could have been due to variation in time period and environmental effects.

Factors affecting persistency: Least squares analysis of variance using mixed model (model-1) was used to study the effect of various non-genetic factors such as parity, season and period of calving, by taking them as the fixed effect. The mean sum of squares of each fixed effect is shown in Table 1. Period of calving is highly significant and least square means of each non-genetic factors of different lactation persistency methods are shown in Table 2.

The effect of lactation order (parity) was not significant (P>0.05) on P1 and P2 measures of persistency (Table 1). The second parity had the highest values of least squares means in P1 and third parity in P2, 2.43±0.15 and -4.19±1.28, respectively (Table 2). Further, no specific trend of decline/rise in persistency was observed for

Table 1. Mean sum of squares of different measures of persistency indices

Source of variation	Degree of freedom	Wood's (P1)	Wilmink's (P2)
Parity	4	0.9721	126.02
Season	3	0.4388	78.31
Period of calving	3	92.101**	833.86**
Error		1.66043	73.8525

** Highly significant (P<0.01) and non-significant (P>0.05).

Table 2. Parity, season and period of calving wise least square means and their standard errors for different measures of persistency indices

Class	No. of observation	Wood's	Wilmink's
Mean	322	2.37 ± 0.08	-5.4 ± 0.56
Lactation order			
1	72	2.35 ± 0.17	-4.55 ± 1.08
2	77	2.43 ± 0.15	-4.41 ± 1.00
3	46	2.09 ± 0.20	-4.19 ± 1.28
4	45	2.01 ± 0.20	-8.05 ± 1.32
5 to 8*	81	2.41 ± 0.16	-4.52 ± 1.06
Season of calving			
Winter	139	2.29 ± 0.11	-5.19 ± 0.75
Summer	95	2.23 ± 0.14	-6.49 ± 0.89
Rainy	47	2.47 ± 0.20	-3.83 ± 1.27
Autumn	40	2.04 ± 0.21	-5.06 ± 1.38
Period of calving			
1996-2002	94	1.02^{a} ± 0.15	-9.66^{a} ±0.96
2003-2008	74	1.76^{b} ± 0.16	-5.51^{b} ±1.04
2009-2013	62	2.56^{c} ± 0 .17	-2.66^{c} ± 1.13
2014-2018	91	3.70^{d} ± 0.15	-2.74^{c} ± 0.97

*The lactation order of 5 to 8 was clubbed together due to lesser records.

different parities. Similar inferences were drawn by Singh and Shukla (1985) in Gir cattle.

The season of calving had no significant (P>0.05) effect on P1 and P2 measures of persistency indices (Table 1). Results resembles the outcomes of Kumar and Singh (2004, 2006) and Kaushal *et al*. (2016). The cows calving in the rainy season were more persistent according to estimate based on P1 (2.47±0.20) and P2 (-3.83±1.27) as in Table 2.

There was significant difference between the summer and winter calvers and also between winter and rainy calvers. More persistency was found in rainy calvers when compared with winter calvers. The abundant green fodder availability, favourable climatic and managemental conditions could have made the rainy calvers more persistent. Sudden decline in milk production after the peak yield in winter calvers could be due to the low ambient temperature and deficiency of nutritive feed. Studies have reported that the winter calvers were least persistent (Kumar and Singh 2004, 2006). Solkner and Fuchs (1987) reported high value of persistency measure in winter season for Simmental cattle.

The effect of period of calving was highly significant (P≤0.01) for P1 and P2 measures of persistency. The present outcomes are in agreement with findings of Tekerli *et al.* (2000), Rekik *et al*. (2003), Kumar and Singh (2004, 2006) and Fadlelmoula *et al*. (2007) in different crossbred and exotic cattle and Kaushal *et al*. (2016) in Sahiwal cattle. In 2014-2018 highest lactation persistency was shown by both P1 and P2. No regular trend in different measures of persistency was observed over the time periods.

Correlation of persistency indices with production traits: The correlation results are depicted in Table 3. Phenotypic correlation between persistency and peak yield (PY) in P1 and P2, were negative and significant only in P1. In Hariana cattle, Gill (1971) reported negative correlation. Positive correlation was reported by Torshiz (2016), Gupta and Johar (1982) in different indigenous and crossbred cattle. Significant positive phenotypic correlation between persistency indices and total milk yield (TMY) was observed. In indigenous breeds, positive correlation was reported by Gill (1971), Shingare *et al*. (2015) and Sachan (2020). Phenotypic correlation between persistency and lactation length (LL) were negative for P1 (-0.02) and P2 (-0.01). On contrary, Shingare *et al*. (2015), Sachan (2020) and Seangjun *et al*. (2009) found positive correlation.

Identification of SNPs: Sequenced data was aligned with *bGH*, *GHR* and *IGF1* genes reference sequence retrieved from ENSEMBL with accession numbers (ENSBTAG00000017220, ENSBTAG00000001335 and ENSBTAG00000011082) for identification of SNPs. The positions of SNP are reported with UCD 1.2 assembly as reference. The comparison of exon 10 and promoter of *GHR* gene revealed a total of four mutations in Tharparkar cattle in exon 10 region (rs721387762, rs523589847, rs110265189, rs109300983). The comparison of exon 1, 3 and promoter region of IGF1 gene showed a mutation in Exon 3 (rs133251968).

PCR-RFLP of bGH gene (intron 3 and exon 5): PCR-RFLP analysis of intron 3, carried out using *Msp*1RE, revealed polymorphic pattern showing three genotypes. TT (328 bp), CC (105 and 223 bp) and CT (105, 223 and 328 bp) as shown in Fig. 1. The SNP C48769565T was found to be polymorphic in Tharparkar. Genotypic frequencies of TT, CT and CC were 0.48, 0.39 and 0.12, respectively. The allelic frequency for T and C was 0.68 and 0.31, respectively. In Sahiwal and Karan Fries cattle polymorphism was already reported by Sachan, (2020) and Kour *et al.* (2018). *Msp*1 polymorphisms were reported by Gorbani *et al*. (2009) in Holstein bulls, Maylinda, (2011) in Grati cattle, Paputungan *et al*. (2013) in Ongole crossbred and Soewandi *et al*. (2021) in Kebumen cattle. Putra *et al*. (2013) reported monomorphism of *Msp*1 restriction site in Aceh breed.

PCR-RFLP analysis of exon 5 was carried out using *Alu*1 RE. *Alu*1-RFLP for exon 5 was found to be

Table 3. Phenotypic correlation between persistency indices and production traits

Production trait	Total milk yield	Peak yield	Lactation length	Wood's	Wilmink's
TMY	1	0.67**	0.71**	0.07	0.005
PY	0.67**	1	0.205**	-0.104*	-0.04
LL	0.71**	0.205**	1	-0.02	-0.01

**Highly significant (p<0.01), *significant (p< 0.05) and non-significant (p> 0.05).

Table 4. Association of lactation persistency with genetic variants in Tharparkar cattle

GH- Msp1		
Genotype	Wood's	Wilmink's
TT	2.87±0.44	-5.7±0.74
CT	2.58±0.88	-6.5±1.4
CC	2.02±0.85	-8.5±1.4
p-value	0.59	0.22
GHR- Alu1		
Genotype	Wood's	Wilmink's
GG	2.14±0.17	-6.18±2.8
AG	2.63±0.35	-6.72±0.60
p-value	0.62	0.93

monomorphic for SNP locus G48118256C which revealed a pattern of CC (52 and 171 bp) genotype in Tharparkar cattle (Supplementary Fig. 1). Jakaria and Noor, (2011) in Bali cattle and Shah *et al*. (2019) in buffalo bulls reported monomorphic sites. Whereas, in Karan Fries (Pal *et al*. 2004), Sahiwal (Sachan 2020 and Satyanarayana 2006), Jersey cattle (Dario *et al*. 2008), HF cattle (Hadi *et al*. 2015 and Mohammadabadi *et al*. 2010) polymorphism has been reported and Ulyanov *et al*. 2021 reported polymorphism with highest milk production for heterozygous genotype in black and white breed.

PCR-RFLP of GHR gene (promoter and exon 10): PCR-RFLP analysis of promoter was carried out using *Stu*1 RE. The locus [32059059] was found to be monomorphic with genotype CC (106 and 332 bp) in Tharparkar cattle (Supplementary Fig. 2). Akad *et al*. (2012) reported polymorphism of *Stu*1 in promoter region of Turkish Grey cattle and Anatolian Red cattle. PCR-RFLP analysis of exon 10 carried out using *Alu*1 RE revealed a polymorphic pattern with nucleotide change at locus A31870017G (rs109300983), showing two genotypes -AA (50,100,191

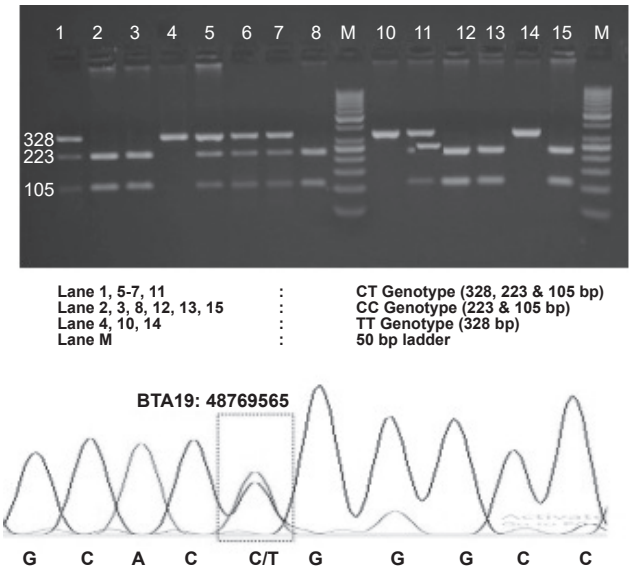

Fig. 1. Resolution of PCR-RFLP product of *GH-Msp*I gene (C48769565T) and chromatogram showing polymorphism in Tharparkar cattle.

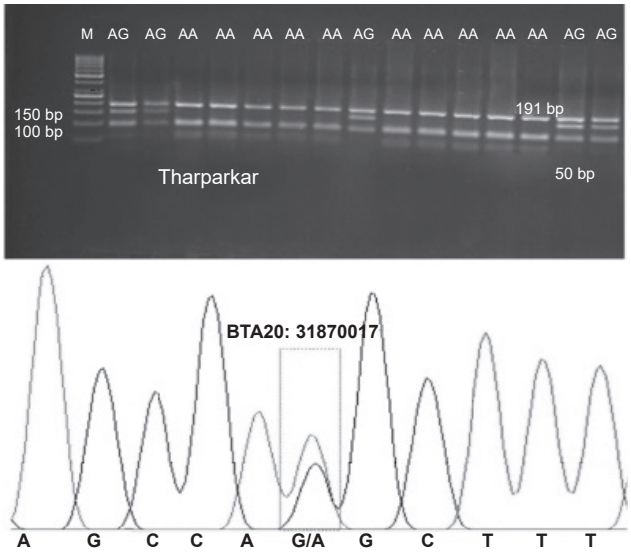

Fig. 2. Resolution of PCR-RFLP of *GHR-Alu*I gene and chromatogram showing polymorphism in Tharparkar cattle.

and 93bp) and AG (150, 191and 93 bp) in Tharparkar cattle with genotypic frequencies of 0.92 (AA) and 0.08 (AG), respectively. The allelic frequency for A and G was 0.96 and 0.04, respectively (Fig. 2).

Deepika and Salar (2013) reported that 79.2% animals were homozygous with AA genotype, 20.8% of animals were heterozygous with AG genotype and they showed that no animals were homozygous for GG genotype. Polymorphism of *Alu*1 in exon 10 was reported by Maj *et al*. (2004), Rahbar *et al*. (2010), Putra *et al*. (2019) and Soewandi *et al*. (2021)

PCR-RFLP of IGF1 gene (exon1, promoter and exon3): PCR-RFLP analysis of exon1 carried out using *Sna*B1 RE revealed a monomorphic pattern with CC (250 bp) genotype at locus C66264359T in Tharparkar cattle. In Pasundan cattle, Putra *et al*. (2013) reported the monomorphism at *SnaB*1 site (Supplementary Fig. 3). Polymorphism of *SnaB*1 was reported by Li *et al*. (2004), Reyna *et al*. (2010), Nicolini *et al*. (2013), Suriaty *et al*. (2010) and Ulyanov *et al*. 2021 in different breeds of cattle.

PCR-RFLP analysis of exon 3 was carried out using *Rsa*1 RE. The locus T66207878C was found to be monomorphic with TT (202 and 142 bp) genotype in Tharparkar cattle (Supplementary Fig. 4). In Bali cattle polymorphism of *Rsa*1 was reported by Maskur *et al.* (2012) and Yurnalis *et al*. (2017) in Pesisir cattle. PCR-RFLP analysis of promoter carried out using *Tas*1 RE revealed monomorphic pattern (CC 146 bp) in Tharparkar cattle (Supplementary Fig. 5). Yurnalis *et al*. (2017) reported polymorphism of *Tas*1 in Pesisir cattle.

Association of genotypes with lactation persistency in Tharparkar cattle: No significant association was found between *bGH-Msp*1 and *GHR-Alu*1 polymorphism and lactation persistency estimated using different lactation persistency methods. Association of persistency indices with genotypes are shown in Table 4.

Each of the non-genetic factors had various levels

of significant effects in different methods of persistency. Persistency had shown positive correlation with all production traits except peak yield. In association study of polymorphic genotypes with lactation persistency in Tharparkar cattle, non-significant association was found between *bGH-Msp*1 and *GHR-Alu*1 variants with estimated lactation persistency. The reason for no significant association between genotypes and lactation persistency indices may be due to lower sample size.

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