Indian Journal of Animal Sciences **91** (10): 851–855, October 2021/Short communication
https://doi.org/10.56093/ijans.v91i10.117218

Genetic polymorphism of four candidate genes in dairy cattle of Kashmir, India

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Received: 12 December 2020; Accepted: 21 August 2021

Keywords: Candidate genes, Dairy cattle, Kashmir, Polymorphism

Milk-yield is a polygenic trait and a number of candidate genes have been reported to affect the milk production traits in cattle, viz. β-lactoglobulin, FMO3, DGAT, BGRH, leptin gene, prolactin gene, Kappa-casein, pituitary transcription factor and α-lactalbumin. Therefore, identifying the polymorphism in such genes has been of interest to gain better understanding of their breeding plans (Dekkers 2004). In general, identifying and validating genetic markers for milk production traits is the initial and crucial step to establish a marker assisted selection system.

Caseins are most abundant proteins representing 80% of total milk protein. Three subtypes of caseins have been identified. α-casein, is subdivided in αs1 and αs2, β-casein and κ-casein (Barreras *et al.* 2001). κ-casein (κ-CN), has been most extensively studied in dairy-milk because it is a casein stabilizer. Fourteen allelic variants have been identified and B allele has been favoured with respect to the A allele because it has been associated to high protein content, positive properties on milk curdling, faster curdling time and increased curd firmness, a larger protein proportion, higher stability upon freezing and larger cheese yield (5–10%) (Fox and Brodkorb 2008).

β-lactoglobulin (β-LG) represents almost 50% of whey protein and 12% of total dairy milk protein. This protein has great nutritive value, solubility and stability and antioxidant capacity, which increases nutritional value. Among the 11 allelic variants, BB genotype of β-GL is related to a high content of milk caseins and an improved cheese yield and larger fat content (Hayes *et al.* 993).

Prolactin (PRL) hormone is a multipurpose hormone (Bole-Feysot *et al.* 1998) which is involved in each stage of milk protein genes expression, i.e. transcription, mRNA stabilization, translation, and post-translational modifications of the proteins. Significant associations of PRl gene with milk production traits have been identified and act as a useful genetic marker in a selection programme of dairy cattle. *Pit-1* gene is a candidate gene for the regulation of growth and development of mammary glands in cattle and other mammals.

Keeping in view the challenge of dairy development in 21st century for a fast-expanding health-conscious human population, and the opportunities offered by science of molecular genetics in animal improvement, the present study was undertaken to check whether or not there was any polymorphism in the above mentioned candidate genes which could be further explored for making informed selection decisions.

The study was undertaken on 120 dairy cows of two genetic groups, viz. Jersey and Crossbred HF (60 each), maintained at an organized dairy farm of Mountain Livestock Research Institute, Manasbal. About 10 ml of blood was collected from each animal and DNA was isolated by standard phenol-chloroform extraction method (Sambrook and Russell 2001). The quality and quantity of the genomic DNA was also done.

Primers for the κ-CN (Borrosa *et al.* 1998), β-LG (Ron *et al.* 1994), PRL (Brym *et al.* 2005) and PIT-1 (Renaville *et al.* 1997) genes were selected from the literature. PCR amplifications of selected genes were carried out in reaction volume of 25 µl in a 200 µl thin-walled sterilized PCR tubes following the conditions given in Tables 1 and 2. The amplified PCR products were checked by horizontal gel electrophoresis. Fast digest enzymes, viz. *Hind III, Hae III, Rsa I* and *Hinf I* were used for polymorphism analysis of the *κ-CN, β-LG, PRL* and *PIT-1* candidate genes. The digested products were visualized under UV light on transilluminator. The gel was recorded in a Gel Documentation System. The banding patterns were scored manually and the numbers of different genotypes were recorded for each gene. The frequency of different genotypes and alleles were calculated by using the standard procedure given by Falconer and Makay (1998) and Popgene 1.31 (Yeh *et al.* 1999) statistical software. The exact test for the deviations from Hardy-Weinberg equilibrium was performed using software Popgene version 1.31 (Yeh *et al.* 1999) to analyse the equilibrium of the population under study.

Polymorphism analysis

A specific single band of length 453 bp, 262 bp, 294 bp and 451 bp were amplified for *κ-CN*, *β-LG*, *PRL* and *PIT1*

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genes, respectively in all the genetic groups under study and were documented in Gel Documentation System (Figs 1, 2, 3 and 4)

κ-CN *gene:* Exon IV region (453 bp) of *κ-CN* gene generated three genotypes which were designated as KK (single band of 453 bp), Kk (three bands 453, 350 and 103 bp) and kk (two bands of 350 bp and 103 bp). In Jersey cows, only two patterns (Kk and kk) and in crossbred cows all the three different (KK, Kk and kk) patterns were found (Fig. 5). Our designated genotypes KK, Kk and kk were same as reported genotypes (AA, AB and BB). Alipanah *et al.* (2008) that observed this in Bos *indicus* and Bos *taurus* and in HF. The absence of KK genotype in Jersey cows is probably due to small sample size that is also reported by Galila and Darwish (2008) in buffalo and HF cows where AA genotype was found to be absent.

β-*LG gene:* A pattern of two bands 153 and 109 were

Table 1. PCR condition for amplification of *κ-CN*, *PRL* and *PIT-1* genes

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

Table 2. PCR condition for amplification of *β-LG* gene

Step	Temperature (°C)		Time
Initial denaturation	95		1 min
Cycling denaturation	95	34 Cycles	30 sec
Annealing	60		90 sec
Cyclic extension	72		2 min
Final extension	72		5 min

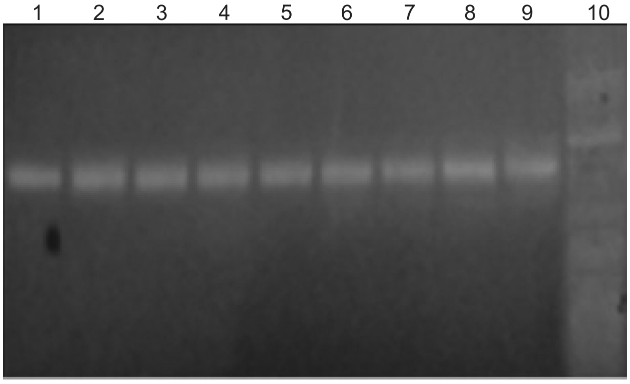

Fig. 1. κ-Casein (κ-CN) gene: 453 bp. Lanes 1–5, Jersey cows; Lanes 6–9, Crossbred cows; Lane 10, 100 bp DNA ladder.

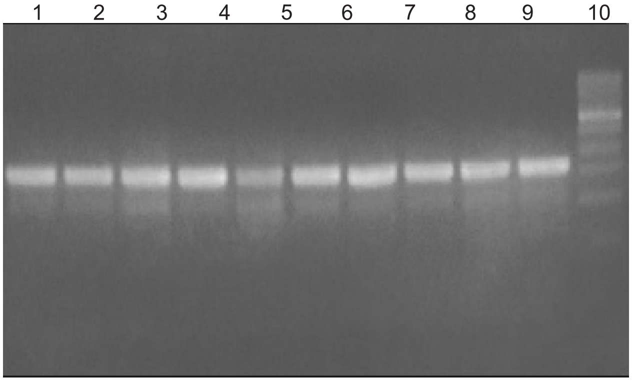

Fig. 2. Beta-Lactoglobulin (*β-LG*) gene: 262 bp. Lanes 1–5, Jersey cows; Lanes 6–10, Crossbred cows; Lane 11, 100 bp DNA ladder.

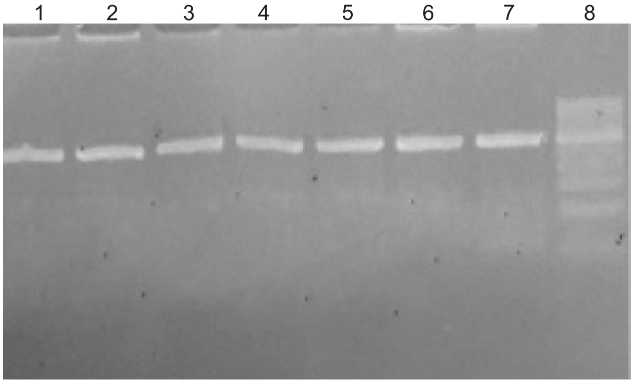

Fig. 3. Prolactin (PRL) gene: 294 bp. Lanes 1–4, Jersey cows; Lanes 5–7, Crossbred cows; Lane 8, 50 bp DNA ladder.

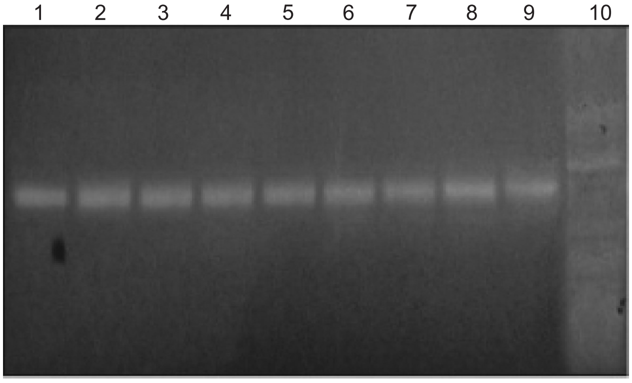

Fig. 4. Pituitary transcription inhibition factor 1 (PIT-1): 451 bp. Lanes 1–4, Jersey cows; Lanes 5–9, Crossbred cows; Lane 10, 100 bp DNA ladder.

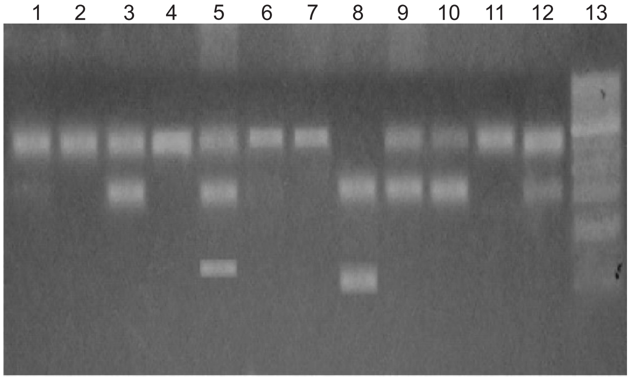

Fig. 5. RFLP *κ-CN* gene with *Hind III* restriction enzyme. Lanes 1–6, Jersey cows (two patterns KK and Kk); Lane 7–12, Crossbred cows (three patterns KK, Kk and kk); Lane 13, 100 bp DNA ladder.

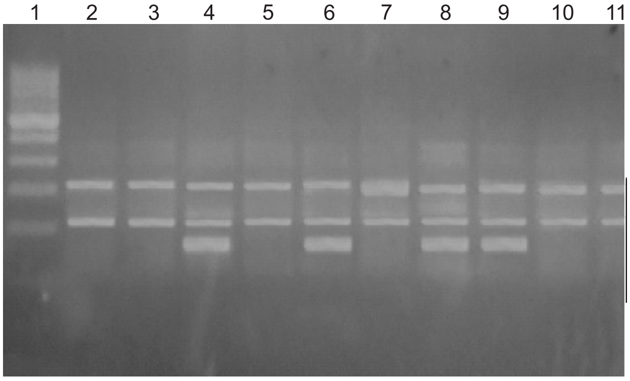

Fig. 6. RFLP BLG gene with *Hae III* restriction enzyme. Lanes 2–6, Crossbred cows (Two patterns Bb and bb); Lanes 7–11, Jersey cows (two patterns Bb and bb); Lane 1, 100 bp DNA ladder.

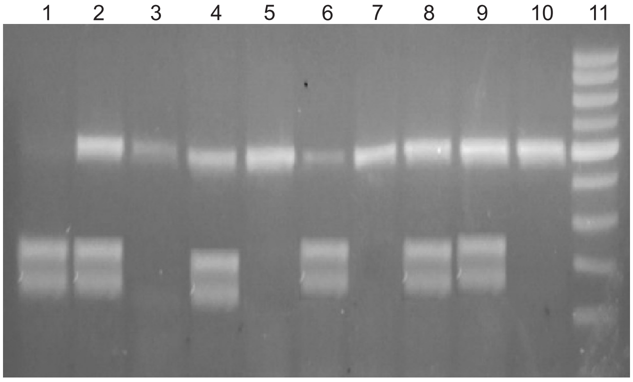

Fig. 7. RFLP PRL gene with *Rsa I* restriction enzyme. Lanes 1–6, Crossbred cows (Three patterns RR, Rr and rr); Lanes 7–10, Jersey cows (two patterns RR and Rr); Lane 11, 50 bp DNA ladder.

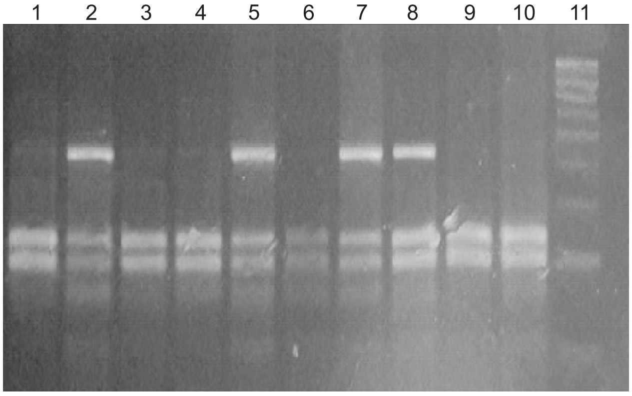

Fig. 8. RFLP PIT gene with *Hinf I* restriction enzyme. Lanes 1–5, Crossbred cows (Three patterns RR, Rr and rr); Lanes 6–10, Jersey cows (two patterns RR and Rr); Lane 11, 100 bp DNA ladder.

designated as bb genotype and with four bands of sizes 153/109/79/74 was designated as Bb genotype (Fig. 6). Bands (79/74 bp) could not be separated thus appeared as a single thick band. All the genetic groups under study showed the same pattern. These findings were consistent with studies of Heidari *et al.* (2009) and Lukac *et al.* (2013).

PRL gene: Three banding pattern viz. RR (single band of 294 bp), Rr (three bands 294 bp, 162 bp and 132 bp) and rr (two bands of 162 bp and 132 bp). In Crossbred cows, all the three patterns were present but in Jersey cows genotype rr was absent (Fig. 7). Similar RFLP patterns were reported by Sacravarty *et al.* (2008) in Kankrej cattle and Ghasemi *et al.* (2009) in Montebeliard cows.

PIT-1 gene: In both genetic groups, only two different RFLP patterns were found with *Hinf I* restriction enzyme (Fig. 8). Accordingly, genotypes were designated as Pp (three bands of 451 bp, 244 bp and 207 bp) and pp (two bands 244 bp and 207 bp). Our results were in contrast with the findings of Ozdemir (2012) showing all the three genotypes. The presence of only two genotypes may be due to smaller population size or due to selection pressure.

Allele and genotype frequencies

κ-CN gene: The heterozygote Kk genotype was most abundant in Jersey cows (0.60). The frequency of K allele was highest in Jersey cows (0.70) and relatively low in crossbred cows (0.53) (Table 3) corroborating several findings observed in *B. indicus* and *B. taurus* by Alipanah *et al.* (2008). The allelic results were also similar to earlier studies reported for Korean native cattle, Japanese brown, Angus, Hereford, Charolais and Holstein cows by Hung *et al.* (1995, 1998). Malik *et al.* (2000) reported similar observations in fourth-generation crossbred cattle. High frequency of A allele was also observed in Brazilin Zebu Gyr, Guzerat and Nelore cattle (Azevedo *et al.* 2008). Bonvillani *et al.* (2010) observed a higher frequency of allele A and lower frequency of allele B in Holstein cows.

Table 3. Genotypic and allelic frequencies of κ-CN, PIT1, PRL and β-LG in Crossbred and Jersey cows

Gene	Genotype	Frequency	Allele	Frequency	Chi-square
κ-CN					
Cross-bred	KK	0.29	K	0.53	0.026
	Kk	0.49	k	0.47	
	kk	0.22			
Jersey	KK	0.40	K	0.70	0.051
	Kk	0.60	k	0.30	P>0.05; NS
PIT-1					
Cross-bred	Pp	0.09	P	0.05	0.199
	pp	0.91	p	0.95	
Jersey	Pp	0.18	P	0.18	0.01
	pp	0.82	p	0.82	P>0.05; NS
PRL					
Cross-bred	RR	0.26	R	0.50	0.002
	Rr	0.50	r	0.50	
	rr	0.24			
Jersey	RR	0.40	R	0.70	0.002
	Rr	0.60	r	0.30	P>0.05; NS
β-LG					
Cross-bred	Bb	0.56	B	0.28	0.032
	bb	0.44	b	0.72	
Jersey	Bb	0.68	B	0.34	0.002
	bb	0.32	b	0.66	P>0.05; NS

Contrary to these findings, Ceriotti *et al*. (2004) reported that allele B has higher frequency than allele A in *B. taurus* breeds as compared to *B. indicus* breeds. Moin and Supriyantono (2012) also compared allele frequencies at kappa casein locus in the breeds of *B. taurus* and reported higher frequency of B alleles than the breeds of *B. indicus.*

Pit1 gene: The pp genotype was found highest in crossbred cows (0.91), followed by jersey cows (0.64). Crossbred cows harbour the highest frequency of p allele (0.95), followed by 0.82 in jersey cows (Table 3). As can be seen in references in terms of Pit-1 polymorphisms (*Hinf*I_451 bp), A allele seems to have a lower frequency value than B allele. This situation is similar to the current study.

PRL gene: The heterozygote Rr genotype was most abundant in jersey cows (0.60) followed by crossbred cows (0.50). The frequency of R allele was highest in jersey cows (0.70) whereas lowest in crossbred cows (0.50) (Table 3). Similar results were reported by Klauzinska *et al.* (2004) in Polish Red and Polish Black and White cattle; Brym *et al.* (2005) in Jersey and Black and White cattle and Skinkyte *et al.* (2005) in Lithuanian Black and White and Lithuanian Red cattle. The results obtained in the present study are not in agreement with those reported in Red Pied cattle, Russian Black Pied and Red Pied cattle and Alipanah *et al.* (2008) in Russian Black Pied and Red Pied cattle.

β-LG *gene:* The heterozygote Bb genotype was most abundant in jersey cows (0.68) and lowest in crossbred cows (0.56). The frequency of b allele was found to be highest in crossbred cows (0.72), whereas lowest in jersey cows (0.66) (Table 3). These findings are consistent with studies of other authors (Daniel *et al.* 2008) who observed that allele A has less frequency than B allele in *B. taurus* as well as in *B. indicus* breeds. In contrast to these findings, Heidari *et al*. (2009) reported allele frequencies of A allele higher than B. This was similar to that demonstrated by Gouda *et al.* (2011) in Egyptian Holstein cattle.

Our results indicated that our population was in Hardy Weinberg equilibrium ($p>0.05$) suggesting no recent selection pressure for any of the alleles studied herein.

SUMMARY

Genetic information is necessary to devise strategic plans aimed to improve the genetic merit of dairy cattle. Exploration of quantitative, qualitative, and molecular genetics is important to improve dairy cattle performance. The aim of the study was to detect the presence of and to identify the polymorphism of kappa-casein (*κ-CN*), beta-lactoglobulin (*β-LG*), prolactin (*PRL*) and pituitary inhibition factor 1 (*PIT-1*) in 120 dairy cows of two genetic groups; Jersey and crossbred HF cows (60 each) maintained at an organized farm in Kashmir. PCR-RFLP of all selected candidate genes revealed ‘KK’, ‘Kk’ and ‘kk’ for HF crossbred cows and ‘KK’ and ‘Kk’ genotypes in Jersey cows for *κ-CN* gene. All animals under study showed 2, viz. Bb and bb genotypes for *β-LG* gene. In crossbred cows, all 3 patterns ‘RR’, ‘Rr’ and ‘rr’ were present but in Jersey cows, the genotype ‘rr’ was absent for *PRL* gene. In the genetic groups under study, 2 genotypes ‘Pp’ and ‘pp’ were found for *PIT*-1 gene. Genotypes Kk, Bb, Rr and pp were most prevalent in our selected population for *κ-CN, β-LG, PRL* and *PIT-1* genes, respectively. Frequencies of K, b, R and p were found to be high for *κ-CN, β-LG, PRL* and *PIT-1* genes, respectively. Chi-square analysis and p-values showed that the population does not vary significantly and proves that the population is in equilibrium. Our populations of Jersey and crossbred Friesian Holstein cattle were polymorphic. Genotypes Kk, Bb, Rr and pp were most prevalent in our population for κ-CN, β-LG, PRL and PIT-1 genes, respectively. The Chi-square and p-values showed that the population does not vary significantly and proves that the population is in equilibrium.

ACKNOWLEDGEMENT

The authors are thankful to SKUAST-K, for providing necessary facilities and support for the present study.

REFERENCES

Alipanah M, Alexandrovna K and Veladimirovich R. 2008. Kappa-casein and PRL-*Rsa* genotypic frequencies in two Russian cattle breed frequencies. *Animal Biotechnology* **57**(218): 131–38.

Barreras A, Robinson O, Monge F J, Vizcarra O and Navarro L H. 2001. Effects of genotypes of casein and serum proteins on milk production in Holsteins. Proceeding, Western Section, *A Society of Animal Science* **52**: 339–411.

Bonvillani A G, Di-Renzo M A and Tiranti I N. 2010. Genetic polymorphism of milk protein loci in Argentinian Holstein cattle. *Genetics of Molecular Biology* **23**(4): 819–23.

Brym P, Kaminski S and Wojcik E. 2005. Nucleotide sequence polymorphism within exon 4 of bovine prolactin gene its associations with milk performance traits. *Journal of Applied Genetics* **45**: 179–85.

Daniel I, Salajeanu A, Magdin A, Stanca C and Vintila I. 2008. Genetic polymorphism at the β-lactoglobulin locus in a dairy herd of Romanian spotted and brown of Maramures. *Animal Husbandry of Biotechnology* **41**(1): 104–07.

Dekkers J C M. 2004. Commercial application of marker and gene associated selection in livestock: Strategies and lessons. *Journal of Animal Science* **82**: 313–28.

Falconer D S and Mackay T F C. 1996. *Introduction to Quantitative Genetics.* 4th edition. CABI, New York.

Fox P F and Brodkorb A. 2008. The casein micelle historical aspects, current concepts and significance. Review. *International Dairy Journal* **18:** 677–84.

Galila A and Darwish F. 2008. A PCR-RFLP assay to detect genetic variants of kappa-casein gene in cattle and buffalo. *Arab Journal of Biotechnology* **11**(1): 11–18.

Ghasemi N, Mohammad Z, Ghodratollah R and Seyed H H. 2009. Associations between prolactin gene polymorphism and milk production in Montebeliard cows. *International Journal of Genetics and Molecular Biology* **1**(3): 48–51.

Gouda E M, Galal M K, Wasfy M A and Abdelaziz S A. 2011. Phenotypes, genotypes and allele frequencies of *β-lactoglobulin* in Egyptian cattle and buffalo. *Journal of Agricultural Science* **3**: 203–10.

Heidari M, Azari M A, Hasani S, Khan A and Zerehdaran S. 2009. Association of genetic variants of *β-lactoglobulin* gene with milk production in a herd and a superior family of Holstein cattle. *Iranian Journal of Biotechnology* **7**(4): 254–57.

Klauzinska M, Zurkowski M, Siadkowska E, Szymanowska M, Grochowska R, Zwierzchowski L and Klewiec J. 2004. Analysis of genetic structure in Polish Red and Polish Black-and-White cattle using twelve marker loci potentially related to milk or meat production traits. *Animal Science Papers and Reports* **22**(2): 153–71.

Lukac D, Vidoviæ V, Nemeš •, Stupar M and Popoviæ-Vranješ A. 2013. Genotypic frequencies of the *β-lactoglobulin*, *κ-casein* and transferrin in Serbian Holstein-Friesian dairy cattle. *Mljekarstvo* **63**(4): 203–10.

Miyai S, Yoshimura S, Iwasaki Y, Takekoshi S, Lloyd R V and Osamura R Y. 2005. Induction of GH, PRL, and TSH beta mRNA by transfection of pit-1 in a human pituitary adenoma-derived cell line. *Cell Tissue Research* **322**: 269–77.

Sacravarty G, Vadodaria V P, Joshi C G, Brahmkshtri B P, Shah R R and Solanki J. 2008. Prolactin gene polymorphism and its association with economic traits in Kankrej cattle. *Indian Journal of Dairy Science* **61**(4): 273–76.

Skinkyte R, Zwierzchowski L, Riaubaite L, Baltrenaite L and Miceikiene I. 2005. Distribution of allele frequencies important to milk production traits in Lithuanian Black and White and Lithuanian Red cattle. *Veterinarija Ir Zootechnika* **31**(53): 93–97.

Yeh F C, Yang R and Boyle T. 1999. POPGENE. Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis, University of Alberta. Edmonton, AB, Canada.