AKADÉMIAI KIADÓ

Acta Veterinaria Hungarica

69 (2021) 4, 324–333

DOI:
10.1556/004.2021.00046

RESEARCH ARTICLE

Single nucleotide polymorphism in *STAT5A* could not endorse variation in milk production traits in Indian bovine population

SUDHAKAR KROVVIDI[1*], THIRUVENKADAN K. ARANGANOOR[2], SARAVANAN RAMASAMY[2] and MURALI NAGARAJAN[2]

[1] Department of Animal Genetics and Breeding, NTR College of Veterinary Science, S.V. Veterinary University, Gannavaram, 521102, Andhra Pradesh, India

[2] Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Received: 4 April 2021 • Accepted: 29 September 2021
Published online: 12 November 2021

ABSTRACT

The Signal Transducer and Activator of Transcription 5A (*STAT5A*) gene involved in activating the transcription of milk protein genes was predicted to be influencing milk production traits. The present study was undertaken to investigate the suitability of the polymorphism of *STAT5A* as a marker for milk traits in Ongole, crossbred cattle and Murrah buffaloes from Southern India. Blood samples (n = 502) for DNA isolation and milk samples (n = 222) from different genetic groups were collected from various farms. The gene variants upon polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) on the exon 7 region of *STAT5A* were subjected to GLM analysis to evaluate their association with milk production traits. The frequencies of *C* and *T* alleles at the *STAT5A*/AvaI locus were 0.98 and 0.02 (Jersey crossbred), 0.94 and 0.06 [Holstein-Friesian (HF) crossbred], 0.97 and 0.03 (Ongole). *T* allele was not observed in Murrah buffaloes. The least squares mean lactation milk yield of *CC* and *CT* genotypes of *STAT5A* were 2,096.90 ± 48.63 and 2,294.41 ± 215.85 kg in Jersey crossbred, 2,312.92 ± 91.01 and 2,392.82 ± 207.66 kg in HF crossbred and 528.40 ± 22.10 and 396.37 ± 76.17 kg in Ongole cattle, respectively. The milk fat content of the *CC* genotype was higher ($P > 0.05$) in Jersey crossbred cattle. The *CT* genotypes of Ongole and HF crossbred cattle recorded a higher fat per cent than the *CC* genotypes. Significant associations were not observed in support of *STAT5A* as a marker for milk production traits in either Ongole or crossbred cattle of indicine admixture and no reason could be found to consider this locus as universal markers for milk production traits in indicine cattle and buffaloes. Considering the monomorphic nature of the gene in buffaloes and their higher milk fat content as compared to bovine milk, much remains to be explored regarding the underlying differences across the bovine and the bubaline species.

KEYWORDS

Ongole cattle, Murrah buffalo, genetic variation, milk yield, fat, SNF

INTRODUCTION

Genetic improvement of indigenous cattle is an integral part of livestock policy towards the increase in milk production, aiming at the protection, conservation and promotion of such breeds. Indigenous cattle of India are highly resilient to heat and adaptive in the tropics, and few of the native breeds are acclaimed around the world and have been used in developing different genetic lines elsewhere (Sanders, 1980; FAO, 2006; Faria et al., 2009; Madalena et al., 2012; Sutarno and Setyawan, 2016). The Ongole (Nelore) cattle breed is one of the familiar dual-purpose breeds in peninsular India, known for its sturdiness, rusticity and adaptability. These cattle were regarded as fair milk producers, yielding 432 ounces (~12.8 L) of milk per day (Shortt, 1889) and were considered as a good breed among the milking animals during the early 1900s (Littlewood, 1936). A shift in the selection priorities of traditional breeders towards the

*Corresponding author.
E-mail: vetsreesudha@rediffmail.com

appearance and gait of these cattle, domestic preferences for buffalo milk, and indiscriminate cross-breeding are a few of the factors that have led to the decline of milk line in this breed. In India, buffalo milk constitutes about 49.2 per cent of total milk production (Report, 2017) and it is the preferred source of milk due to its taste and higher fat content; thus, it is fetching higher prices at the market. However, over the years buffaloes have received relatively little attention and much of their genetic potential is yet to be exploited.

The rapid improvement in traits of economic importance in livestock species embarks on identification of the underlying genes and their polymorphisms. Over the previous decades many candidate genes with different functions in metabolism, including Signal Transducer and Activator of Transcription 5A (*STAT5A*), have been proposed as affecting milk yield and composition in dairy cattle (Ogorevc et al., 2009). Since STATs are factors that mediate transcription of milk proteins in response to prolactin (Wakao et al., 1994) and are involved in the lactogenic hormone response, the genes coding for these proteins are supposed to influence milk protein and milk production traits in cattle and buffaloes. Polymorphisms in *STAT5A* were previously shown to be associated with milk production traits in certain genetic groups of cattle (Selvaggi et al., 2009; He et al., 2012). However, such studies on Zebu cattle in the tropics and subtropical habitats were scanty. Replication of the reported associations in an independent population is vital to validate the claim of candidate genes. Owing to the physiological role of the *STAT5A* gene, the present study was conducted to determine the polymorphisms and their association with milk production traits in different bovine genetic groups that are popular in Southern India. The geographical area examined in the study includes the Southern plateau region, the East coast plain and hill region and the West coast plain and hill region. These regions are affected by different agro-climatic conditions as shown in Fig. 1.

MATERIALS AND METHODS

Ethics approval

This study did not involve any animal testing or experimental studies with animals and was approved by the

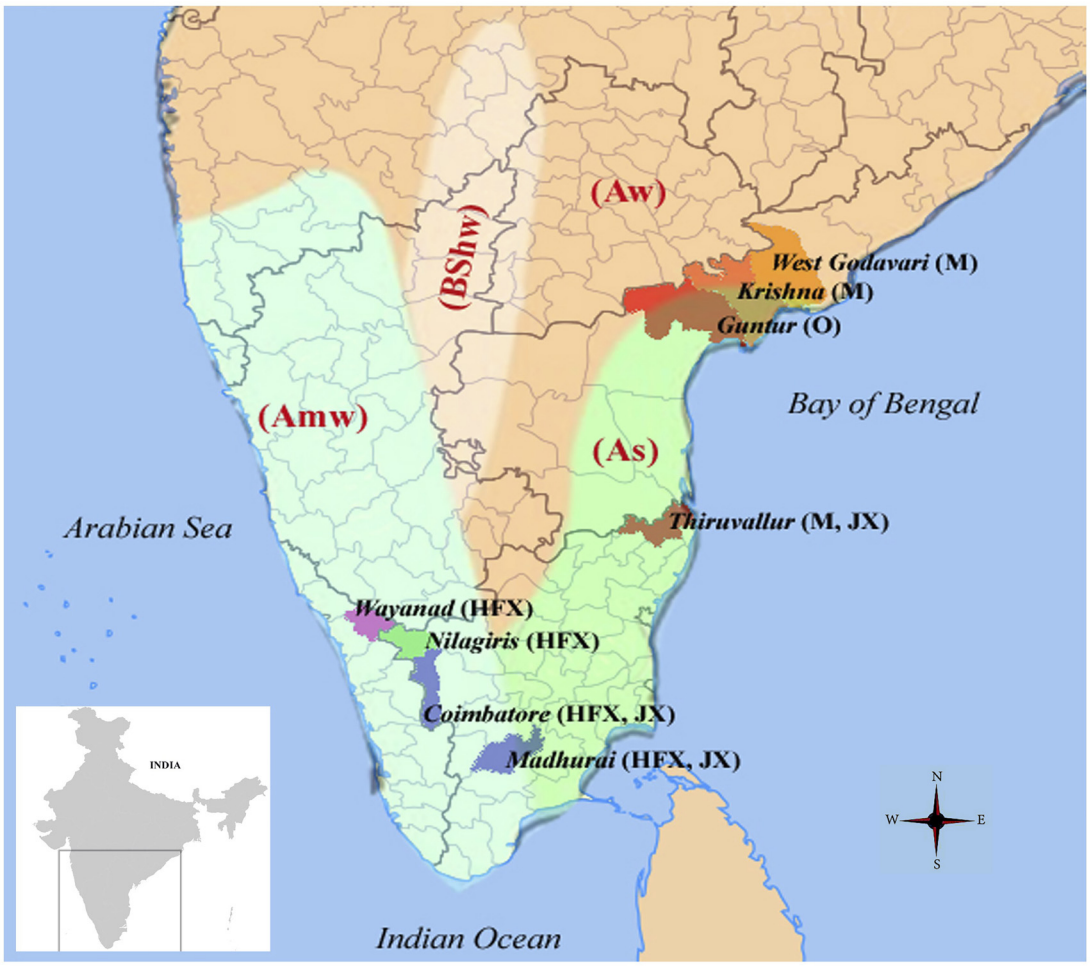

Fig. 1. The sampled bovine population across different agro-climatic regions of Southern India. Climatic zones (Koeppen's classification): Aw – tropical savanna type, BShw – steppe climate, Amw – monsoon type with short dry season, As –monsoon with dry season in summer. Cattle genetic groups: M – Murrah buffaloes, O – Ongole, JX – Jersey crossbred, HFX – Holstein-Friesian crossbred

institutional competent authority (No. 389/PG/AGB/VCRI). The blood samples were collected aseptically from live animals after the written informed consent from the rightful owners of the animals by a qualified veterinarian following all applicable guidelines for the care and use of animals.

The animals utilised in the present study were neither procured nor housed for any specific experimentation but were approached in their rearing habitat. The blood samples (5 mL) were collected aseptically from the jugular vein of each animal at the barn. Whole blood samples (n = 502) from the unrelated individuals belonging to Ongole (Nelore) (135), Holstein-Friesian crossbred (114) and Jersey crossbred (96) cattle, and Murrah buffaloes (157) reared in different organised farms of Tamil Nadu, Andhra Pradesh and Kerala states across Southern India (Fig. 1) were used for genomic DNA isolation (Miller et al., 1988). The native cattle breeds of the area are low or moderate milk producers and are either dual (milk and draft) or draft purpose, compared to the breeds with their home tract in the Northern part of the country. The cattle included in the study were from an inter-se mated crossbred population that resulted from using half-bred bull semen following the National Cattle Breeding Policy. A previously identified PCR-RFLP marker on *STAT5A*-exon 7 (C→T) (Flisikowski et al., 2003) was chosen and the genomic region was amplified by modifying the protocol and performing PCR in a reaction volume of 15 µL comprised of PCR mastermix (2x Taq Master Mix Red, Amplicon, Denmark) along with 5 pmol each of forward and reverse primer. The cycling conditions for *STAT5A* amplification included denaturation at 94 °C/2 min, followed by 34 cycles: 94 °C/1 min, 60 °C/1 min, and 72 °C/1 min. The final extension was 72 °C/3 min. The amplicon of each sample (10 µL) was digested with 10 units of AvaI (Eco 881) for 6 h, 37 °C to genotype each animal with respect to *STAT5A*. All the digested samples were electrophoresed in agarose gel (Himedia, India) and visualised with Gel Doc™ XR+ (Biorad, USA) to determine genotypes. Evaluation of Hardy–Weinberg equilibrium was performed using the POPGENE 32 (Version 1.32) program by Yeh et al. (2006). The amplicons exhibiting RFLP were further confirmed by sequence-based typing (PCR-SBT) and the sequence variations were compared by Multiple Sequence Alignment Software (DNASTAR, USA). The Neighbour-Joining phylogenetic tree was constructed using the MEGAX program (Kumar et al., 2018).

Out of the total number of animals that were sampled for DNA isolation, milk samples were collected from 222 animals that were in lactation at the time of blood sample collection. Fat, protein, lactose and solids not fat (SNF) contents of milk were determined in fresh samples using Lactoscan SL 30, MB Ver.60 (Ktpl Instruments, India) for association studies. The data on lactation milk yield was collected from the registers maintained at the farms. Additional information on parity, farm location, and lactation milk yield was also collected. Adjustment for the seasonal influences was imperative, so the months were grouped into four seasons to have less intra-group differences than between the groups (Winter: January–February; Summer: March–May; South-West Monsoon: June–September; North-East Monsoon: October–December). The lactations were grouped into three stages: 5–90 days (1), 91–180 days (2), and 181 days and above (3).

Evaluation of the effect of polymorphic variants of the *STAT5A* on lactation milk yield was performed using the General Linear Model procedure (SPSS Statistics base 17) with the following model for lactation milk yield: $Y_{ijklm} = \mu + f_i + s_j + p_k + g_l + (g \times p)_{lk} + e_{ijklm}$, where, Y_{ijklm} = the m^{th} observation of i^{th} farm, j^{th} season, k^{th} parity, l^{th} genotype and interaction of l^{th} genotype × k^{th} parity, μ = overall mean, f_i = fixed effect of i^{th} Farm, s_j = random effect of j^{th} season, p_k = effect of k^{th} parity, g_l = effect of l^{th} genotype, $(g \times p)_{lk}$ = effect of interaction of l^{th} genotype and k^{th} parity, e_{ijklm} = random errors, assumed to be NID (O, σ^2_e). The model employed for the analysis of milk constituents was $Y_{ijkl} = \mu + f_i + p_j + s_k + g_l + (g \times p)_{lk} + e_{ijkl}$, where, Y_{ijklm} = analysed trait, μ = overall mean, f_i = fixed effect of i^{th} farm, p_j = effect of j^{th} parity on the test day, s_k = effect of j^{th} stage of lactation, g_l = effect of l^{th} genotype, $(g \times p)_{lj}$ = effect of interaction of l^{th} genotype and j^{th} parity, e_{ijkl} = random errors, assumed to be NID (0, σ^2_e).

RESULTS

Polymorphism and population indices

The DNA fragment of the *STAT5A* (215 bp) gene in all the three genetic groups was amplified and the polymorphisms observed upon RFLP (Fig. 2). *STAT5A*/AvaI digestion resulted in the *C* allele, restricted by the enzyme into a single position (181 bp), while the *T* allele remains uncut due to the modification of the restriction enzyme site through the *C→T* substitution. Typing allelic variation on Ongole, Jersey crossbred and Holstein-Friesian (HF) crossbred cattle resulted in only two genotypes for the *C/T* polymorphism, viz. *CT* and *CC*. Abundance of the *CC* genotype and absence of the *TT* genotype was observed in all the genetic groups of cattle. The gene frequency of the *C* allele was 0.98 in Jersey crossbred, 0.94 in HF crossbred and 0.97 in Ongole (Table 1).

The analysis on *STAT5A* loci revealed that the present populations were consistent with the Hardy–Weinberg equilibrium ($P > 0.05$). The difference in genotypic frequencies between the cattle genetic groups (Table 2) was significant for the polymorphisms at the *STAT5A*/AvaI locus. Jersey crossbred differed ($P < 0.05$) from the HF crossbred and Ongole, where the differences were not significant ($P > 0.05$) for the HF crossbred with the Ongole breed and also, the allele frequencies of both crossbred cattle did not differ significantly from those of the Ongole breed ($P > 0.05$). The distribution of *STAT5A* genotypic frequencies in all the genetic groups studied revealed heterozygosity values almost equal to the expected heterozygosity (Table 3). The genetic diversity analysis in the examined populations demonstrated that the Jersey crossbred had the highest homozygosity with respect to *STAT5A* loci (0.969) and a low PIC (0.038).

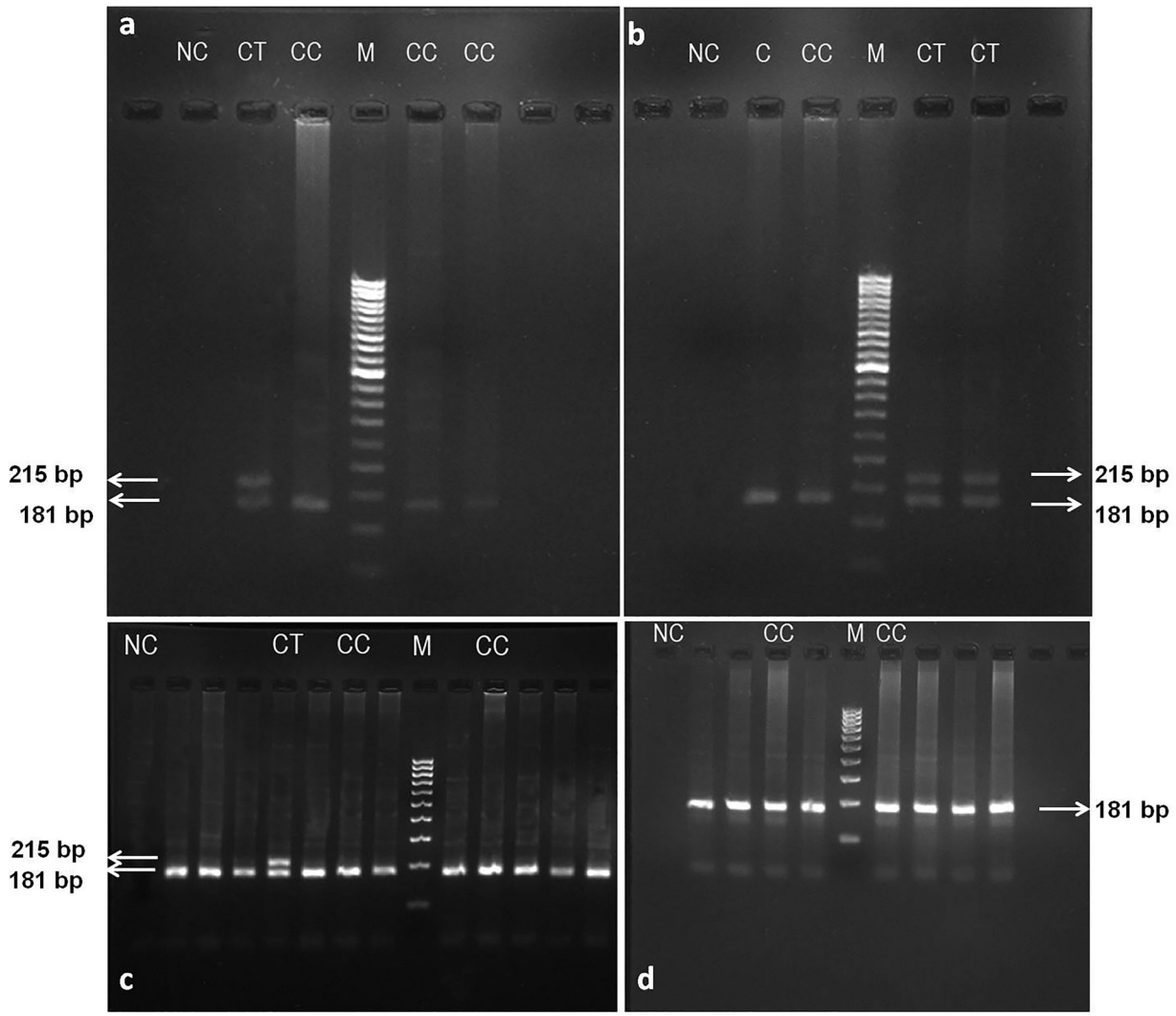

Fig. 2. RFLP pattern of the *STAT5A*/AvaI gene in cattle and buffaloes. (a) Jersey crossbred, (b) Holstein-Friesian crossbred, (c) Ongole cattle, (d) Murrah buffaloes. Genotypes: TT (215 bp), CT (215, 181 and 34 bp) and CC (181 and 34 bp); M: 50 bp DNA marker (a & b), M: 100 bp DNA marker (c & d), NC: negative control

Table 1. Distribution of genotypes and allele frequencies at the *STAT5A*/AvaI locus in cattle and buffaloes

Breed/Group	Number of animals (n)	Observed genotypic frequency			Allele frequency		Expected genotype frequency			χ^2 value	*P* value
		CC	*CT*	*TT*	*C*	*T*	*CC*	*CT*	*TT*		
Jersey crossbred	96	0.97 (93)	0.03 (3)	0	0.98	0.02	0.97 (93.02)	0.03 (2.96)	0 (0.02)	0.02^{NS}	0.90
Holstein-Friesian crossbred	114	0.88 (100)	0.12 (14)	0	0.94	0.06	0.88 (100.40)	0.12 (13.20)	0 (0.40)	0.45^{NS}	0.50
Ongole	135	0.95 (128)	0.05 (7)	0	0.97	0.03	0.95 (128.08)	0.05 (6.84)	0 (0.08)	0.08^{NS}	0.78
Murrah buffalo	157	1.0 (157)	0	0	1	0	–	–	–	–	–

Figures in parentheses are the number of animals, NS: Not significant ($P > 0.05$).

Sequence analysis

The PCR amplicons pertaining to different genotypes were subjected to sequencing. After submission of the sequences to GenBank, accession numbers were obtained for various genotypes of *STAT5A* (Ongole: KJ461324.1 and KJ461325.1, crossbred cattle: KM052367.1 to KM052370.1, Murrah buffalo: KM052371.1). A dinucleotide substitution of

Table 2. Genetic distance based on genotypic and allelic frequencies at the *STAT5A*/AvaI locus

Breed	*STAT5A*/AvaI locus		
	Jersey crossbred	Holstein-Friesian crossbred	Ongole
Jersey cross	–	5.87* ($P < 0.02$)	4.03* ($P < 0.04$)
HF cross	5.62* ($P < 0.01$)	–	0.58 ($P < 0.45$)
Ongole	2.192 ($P < 0.14$)	0.797 ($P < 0.37$)	–

Above the diagonal: χ^2 (P value) for differences in genotypic frequencies (df = 2) between the two breeds.
Below the diagonal: χ^2 (P value) for differences in allelic frequencies (df = 1) between the two breeds.
* Significant difference ($P < 0.05$), ** Significant difference ($P < 0.001$).

Table 3. Heterozygosity statistics and genetic diversity at the *STAT5A* locus

Breed	Gene	Observed homozygosity	Observed heterozygosity	Expected homozygosity	Expected heterozygosity	Ne	PIC	F_{IS}
Jersey crossbred	*STAT5A*	0.9688	0.0312	0.9691	0.0309	1.03	0.038	–0.0159
Holstein-Friesian crossbred	*STAT5A*	0.8772	0.1228	0.8842	0.1158	1.13	0.106	–0.0654
Ongole	*STAT5A*	0.9481	0.0519	0.9493	0.0507	1.05	0.057	–0.027

Ne = effective number of alleles; PIC = polymorphic information content; F_{IS} = fixation index.

CT>GC at 6,852 and 6,853 was observed in all the native and crossbred cattle and buffalo populations from the subcontinent. An additional SNP (G>T) at three nucleotides upstream of the AvaI restriction site in all the animals, and (C>T) at nine nucleotides upstream was observed in crossbred cattle and buffaloes, but not in the Zebu cattle.

Association of polymorphism with milk production traits

Milk yield. The least-squares mean of lactation milk yields in different genetic groups of cattle after adjusting for the effects of farm, season and parity are presented in Table 4. Among the factors studied, only the location of farm had a significant effect ($P < 0.05$) on lactation milk yield. Since no *TT* genotype was observed at the *STAT5A* locus in any of the genetic groups studied, only *CC* and *CT* were included in the association analysis. The mean milk yields of *CC* and *CT* genotypes were 2,096.90 ± 48.63 and 2,294.41 ± 215.85 kg in the Jersey crossbred. The mean milk yields over all the three lactations in the HF crossbred were 2,312.92 ± 91.01 and 2,392.82 ± 207.66 kg for the *CC* and *CT* genotypes, respectively. Although the average milk yield of the *CT* genotypes was higher than that of the *CC* genotypes, the differences were not significant ($P > 0.05$).

Milk composition. The least-squares mean for the milk constituents of *STAT5A* genotypes of Jersey crossbred (72), HF crossbred (50) and Ongole (62) cattle are presented in Table 5. The fat, SNF, protein and lactose content of milk of the *CC* genotype is higher (4.31 ± 0.39, 8.94 ± 0.14, 3.27 ± 0.05 and 4.90 ± 0.08%, respectively) than those of the *CT* genotype in Jersey crossbred cattle, whereas the *CT* genotypes showed higher values in the HF crossbred. In Ongole cows, the values of *CC* and *CT* were almost similar except for fat content, where *CT* genotypes recorded a higher fat percentage (4.36 ± 0.58) than *CC* genotypes (3.52 ± 0.17). The differences were not significant for any of the milk constituents ($P > 0.05$). In the present study, differences were not significant in fat, SNF, protein and lactose contents between the *CC* and *CT* genotypes in either the Jersey crossbred or the HF crossbred.

DISCUSSION

The C→T substitution in exon 7 was determined previously by Dario et al. (2009) in Italian Friesian, Jersey and Podolica breeds, by Sadeghi et al. (2009) in Iranian Holstein, by Selvaggi et al. (2009) in Italian Brown and Romanian Simmental, by Kmiec et al. (2010) in Holstein and by Dario and Selvaggi (2011) in Jersey cattle. In conformity with the present observation in Zebu cattle and their crosses, *TT* genotypes were not reported in dairy breeds of Iranian Holstein bulls and Polish Holstein-Friesian by Sadeghi et al. (2009), Bao et al. (2010) and Kmiec et al. (2010). The

Table 4. Least-squares mean (±SE) of lactation milk yield (kg) in different genetic groups of cattle for *STAT5A* genotypes

	n	Jersey crossbred	*n*	Holstein-Friesian crossbred	*n*	Ongole
Overall mean		2,195.66 ± 110.50		2,352.0 ± 117.93		462.38 ± 39.83
CC	139	2,096.91 ± 48.63	121	2,312.92 ± 91.01	142	528.40 ± 22.10
CT	6	2,294.41 ± 215.85	20	2,392.82 ± 207.66	12	396.37 ± 76.17

n = number of observations. Means with at least one common superscript within classes do not differ significantly ($P > 0.05$).

Table 5. Least-squares mean (±SE) of milk constituents for *STAT5A* genotypes in different genetic groups of cattle

Main effect/ subclass	Jersey crossbred (72)					Holstein-Friesian crossbred (50)					Ongole (62)				
	n	Fat %	SNF %	Protein %	Lactose %	*n*	Fat %	SNF %	Protein %	Lactose %	*n*	Fat %	SNF %	Protein %	Lactose %
Overall mean		3.84 ± 0.48 **	8.90 ± 0.17 *	3.26 ± 0.06 *	4.88 ± 0.09 **		4.24 ± 0.26 *	9.22 ± 0.21 **	3.35 ± 0.07 **	5.06 ± 0.11 **		3.80 ± 0.23	9.35 ± 0.15	3.48 ± 0.10	5.13 ± 0.08
Farm															
1	11	1.88^{a} ± 0.77	8.09^{a} ± 0.27	2.96^{a} ± 0.10	4.44^{a} ± 0.15	4	2.78^{a} ± 0.79	8.29^{a} ± 0.62	3.03^{a} ± 0.23	4.44^{a} ± 0.32	62	3.80 ± 0.23	9.35 ± 0.15	3.48 ± 0.10	5.13 ± 0.08
2	20	5.61^{b} ± 0.74	8.90^{b} ± 0.26	3.26^{b} ± 0.10	4.87^{b} ± 0.14	6	4.50^{b} ± 0.68	9.96^{b} ± 0.53	3.63^{b} ± 0.20	5.46^{b} ± 0.28					
3	16	3.89^{b} ± 0.91	9.60^{b} ± 0.32	3.51^{c} ± 0.12	5.27^{c} ± 0.18	13	4.14^{b} ± 0.44	9.35^{b} ± 0.35	3.43^{b} ± 0.13	5.32^{b} ± 0.18					
4	25	3.99^{b} ± 0.70	8.99^{b} ± 0.25	3.29^{b} ± 0.09	4.93^{b} ± 0.14	18	4.91^{b} ± 0.44	10.14^{c} ± 0.34	3.70^{b} ± 0.13	5.59^{a} ± 0.18					
5		–	–	–	–	9	4.87^{b} ± 0.51	8.36^{b} ± 0.40	2.98^{c} ± 0.15	4.48^{b} ± 0.21					
Genotype															
CC	69	4.31 ± 0.39	8.94 ± 0.14	3.27 ± 0.05	4.90 ± 0.08	41	3.98 ± 0.28	9.16 ± 0.22	3.33 ± 0.08	5.05 ± 0.12	57	3.52 ± 0.17	9.35 ± 0.12	3.49 ± 0.08	5.13 ±0.06
CT	3	2.46 ± 1.66	8.77 ± 0.58	3.21 ± 0.21	4.82 ± 0.32	9	4.76 ± 0.54	9.34 ± 0.42	3.41 ± 0.15	5.07 ± 0.22	5	4.36 ± 0.58	9.34 ± 0.38	3.47 ± 0.26	5.12 ±0.21

n = number of observations; SNF = solids not fat. Means with at least one common superscript within classes do not differ significantly ($P > 0.01$), * $P < 0.05$, ** $P < 0.01$.

absence of the *TT* genotype in the studied populations which were reared for milking purpose concurs with their observations. However, some of the cattle reared for meat purpose, i.e. Charolaise, Limousine and Red Angus, Hereford and Simmental, were also without the *TT* genotype (Flisikowski et al., 2003). Contrary to the present findings, Dario et al. (2009) reported the absence of the *CC* genotype in Italian Friesian. Much lower allele frequencies of *C* were observed in Anatolian cattle native to Turkey (Arslan et al., 2015). The differences in the allele frequency between the populations investigated and those reported by other authors could be due to inter-crossing through artificial insemination of *Bos taurus* with indigenous cattle in the subcontinent.

The analysis of data from Ongole cattle revealed that the *C* and *T* allele frequencies were 0.97 and 0.03, respectively. No studies of *STAT5A* polymorphism in *Bos indicus* breeds could be found in the available literature except for that of Flisikowski et al. (2005) who studied polymorphism at a locus in exon 1 in Dwarf Zebu of Sri Lankan origin and reported fixation of the *A* allele at –226 position. However, polymorphism in a different exon was reported in Zebu cattle from Sudan by Hummeda et al. (2014). Fixation of allele *C* was reported (Paramitasari et al., 2015) in Bali cattle (*B. javanicus*) indigenous to Indonesia, which share a common phenotype with the *B. indicus* breeds in having a hump.

In Murrah buffaloes, the restriction enzyme digestion on *STAT5A* exon 7 yielded no polymorphic pattern and only the undigested product representing the *CC* genotype was observed in the analysed herds. The *C* allele was fixed in the population of the Murrah. A similar observation was reported in Anatolian buffaloes by Daldaban et al. (2020). No studies of *STAT5A* polymorphism at this locus in buffaloes of Indian origin could be found in the literature. SNPs in other regions of *STAT5A* than the presently studied exon 7 were reported in Binlangjiang and Italian buffaloes (Min et al., 2013; Coizet et al., 2018).

The genotype frequency of Jersey crossbred cows significantly differed ($P < 0.05$) from that of HF crossbred and Ongole, but the differences in genotype and allelic frequency were not significant ($P > 0.05$) between the HF crossbred and the Ongole breed (Table 2). The differences between the Jersey crossbred and the HF crossbred suggest a significant effect of breed selection on molecular characteristics of the *STAT5A* gene. The present values (PIC <0.25) are indicative of a good homologous state of Jersey crossbred cattle. The HF crossbred (PIC = 0.106) could be considered as a reliable source of genetic variability for *STAT5A* (Table 3).

The sequences of *STAT5A* of the different genetic groups of the present study were aligned, annotated and multiple sequence analysis was performed with the sequence available (AJ237937.1) in GenBank for SNP detection. A substitution C→T at position 6,853 within exon 7 was reported earlier (Flisikowski et al., 2003; Selvaggi et al., 2009) in taurine cattle. However, the same was not observed in any of the examined genetic groups. Interestingly, compared to the

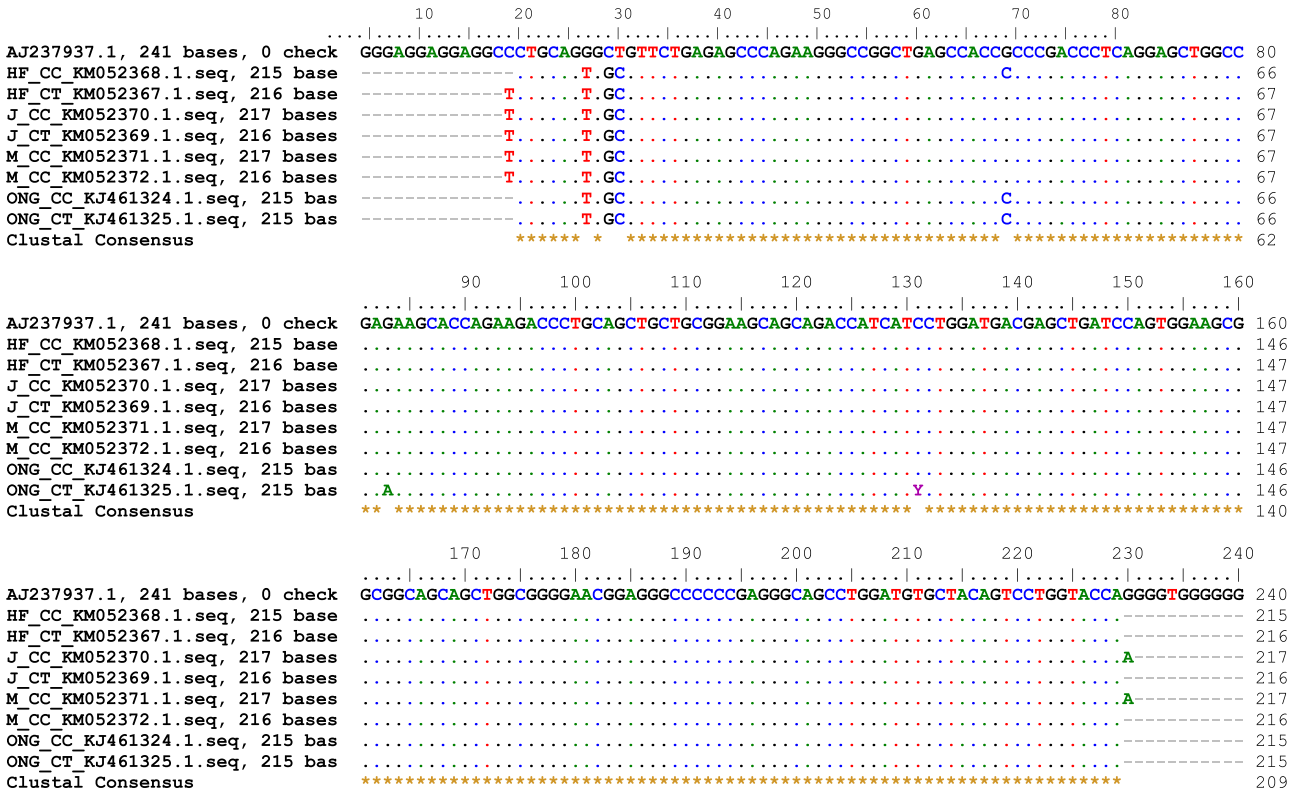

Fig. 3. Variations in STAT5A nucleotide sequence compared with the reference sequence

reference sequence and those available in dbSNP from Ensembl a dinucleotide substitution of CT>GC at 6,852 and 6,853 was observed in all the native and crossbred cattle and buffaloes included in the study (Fig. 3). The SNP (C>T) at nine nucleotides upstream was observed in crossbred cattle and buffaloes but not in Zebu cattle. A variation, G→C transfusion (rs715052038) at 32 nucleotides downstream was observed in Ongole cattle. The absence of C>T and the presence of G>C is unique to Ongole cattle. These differences were also reflected in the phylogenetic tree (Fig. 4), each genetic group reflecting in a single clade showing a monophyletic group of Ongole, Jersey crossbred and HF crossbred. Separation of a clade of HF crossbred from Ongole sequence may be due to the admixture of Ongole cattle with cattle of Holstein-Friesian origin that could be a result of probable indiscriminate crossbreeding (Basu, 2009; Manomohan et al., 2021).

In the present study, the milk yield of *CT* genotypes was superior to that of the *CC* genotypes by 197.51 kg in Jersey crossbred and 79.9 kg in HF crossbred. A similar observation of higher yields by *CT* genotype was reported by Flisikowski et al. (2004) in Polish Friesian cows; however, that comparison was made with *TT* genotypes as no *CC* genotypes were observed in their study. Contrarily, Selvaggi et al. (2009) and Dario and Selvaggi (2011) reported significantly higher milk yield by *CC* genotypes than *CT* genotypes in Italian Brown and Jersey breeds, respectively. The non-significantly higher milk yield by *CT* genotypes than *CC* in the present study was in agreement with reports on taurine cattle by Sadeghi et al. (2009), Kmiec et al. (2010) and He et al. (2012). Contrary to the observations in crossbred cattle, the average milk yield of *CC* genotype was higher than that of the *CT* genotype in Ongole cows, but the differences were not significant ($P > 0.05$). The corresponding values were 528.40 ± 22.10 and 396.37 ± 76.17 kg, respectively. Taken together, from these results it appears that the genotypes do not have a significant effect on milk yield in either crossbred or Ongole cattle. The milk yield in Murrah buffaloes in the first three lactations was found to be 1,737.96 ± 65.55, 1,910.64 ± 55.09 and 1,984.17 ± 137.22, respectively. Since Murrah buffaloes are monomorphic at the investigated loci, an association analysis was not performed.

The higher fat, SNF and protein per cent for *CC* genotypes in Jersey crossbred cattle is in agreement with the reports of Sadeghi et al. (2009), Kmiec et al. (2010), Dario and Selvaggi (2011) and Bao et al. (2010), who reported a non-significantly higher value for *CC* genotypes for this trait. However, Selvaggi et al. (2009) reported significant effects of genotype on these traits. The non-significantly ($P > 0.05$) higher value of lactose content for the *CC* genotype observed in the study was in accordance with the findings of Dario and Selvaggi (2011). They reported that *CC* cows produced more milk than *CT* cows and the protein content was higher, whereas in the present study the *CT* genotype had a higher lactation milk yield in both crossbred populations. Brym et al. (2004) reported a significant influence of the SNP in intron 9 of the *STAT5A* gene on milk fat and milk protein

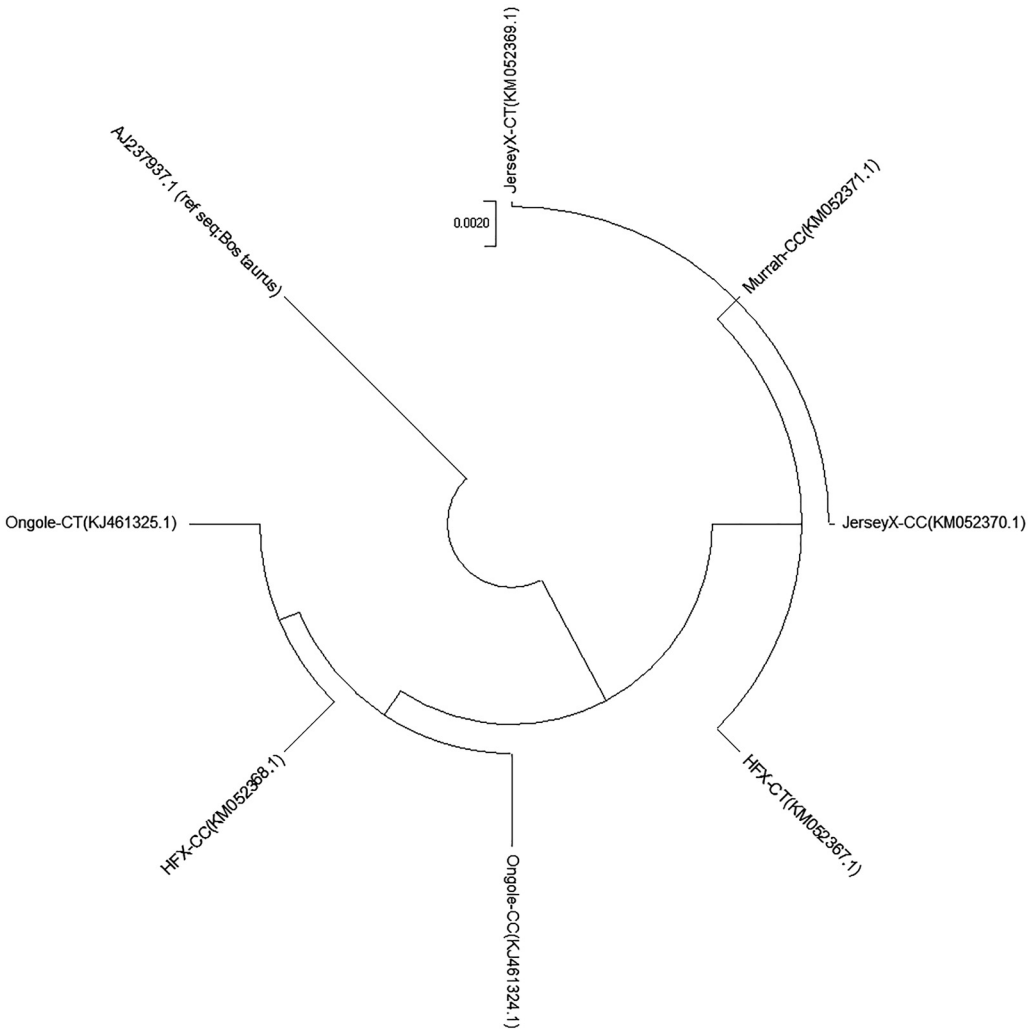

Fig. 4. Phylogenetic tree analyses of the STAT5A exon 7 of zebu, taurine crossbred cattle and buffalo

percentage in Jersey cattle. Regarding most of the milk constituent percentages of *CC* and *CT* in Ongole cattle, no significant inferences could be drawn by comparing the genotypes because the values were almost similar except for the fat per cent. Nevertheless, the superiority of allele *C* over *T* could not be significantly established due to the absence of *TT* genotypes in all the studied genetic groups and could not be included in the statistical analysis for an accurate estimation due to the relatively small number of the sampled animals. In Murrah buffaloes ($n = 38$) the milk fat, protein, SNF and lactose content was 6.52 ± 0.40, 3.47 ± 0.05, 9.29 ± 0.14 and 4.93 ± 0.08%, respectively.

Milk protein gene expression in mammary epithelial cells is regulated by the action of prolactin mediated through the *STAT5A* protein. The exon 7, together with exons 8–12, codes for amino acids 250–480 of the *STAT5A* molecule in the DNA-binding domain responsible for binding to promoters of target genes (Pellegrini and Dusanter-Fourt, 1997). Mutations in this region might result in changes in the transactivation properties of the *STAT5A* protein, and thus influence the level of expression of the genes. The PCR-RFLP variant studied in the present study is a synonymous variant. Hence, the mutation might not have involved changes in the transactivation properties of the *STAT5A* gene. The association observed in the earlier studies could be a linked association to SNP at other loci rather than a direct effect.

The differences observed in the present study from those of the previous studies could be due to the populations in question that are bred under different conditions and for different purposes, and also might be due to differences in the factors incorporated in the model for analysis. Uniform selective pressure on different breeds, for milk and meat production traits, can result in changes in allele frequency in other genomic regions due to polygenic influence and also the pleiotropy that cannot be ignored. The present study could not find a reason to consider this locus as a universal marker for milk production traits in the *indicine* cattle and buffaloes. Each breed retains a unique signature of its selection history and it can be understood that for the dual-purpose breeds, strong selection has not been performed on either milk or beef traits. Considering the monomorphic nature of the gene in buffaloes and their higher milk fat content compared to cattle, much remains to be explored

regarding the underlying differences across the bovine and the bubaline species.

ACKNOWLEDGEMENTS

This research did not receive any specific funding. The authors acknowledge the facility provided by Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. We are thankful to the authorities of TN Agricultural University, Coimbatore; SV Veterinary University, Tirupati, Andhra Pradesh and College of Veterinary Science, Pookote, Kerala and other dairy farmers for according the necessary permissions to collect the required samples and data from their livestock farms. The authors acknowledge the late Dr. S. Panneerselvam, Professor and Head, Department of Animal Genetics and Breeding, Veterinary College and Research Institute (TANUVAS), Namakkal for his support and guidance throughout the study.

REFERENCES

Arslan, K., Akyüz, B. and Agaoglu, O. K. (2015): Investigation of *STAT5A, FSHR*, and *LHR* gene polymorphisms in Turkish indigenous cattle breeds (East Anatolian Red, South Anatolian Red, Turkish Grey, Anatolian Black, and Zavot). Russ. J. Genet. **51**, 1088–1095.

Bao, B., Zhang, C., Fang, X., Zhang, R. F., Gu, C., Lei, C. and Chen, H. (2010): Association between polymorphism in STAT5A gene and milk production traits in Chinese Holstein cattle. Anim. Sci. Pap. Rep. **1,** 5–11.

Basu, P. (2009): Success and failure of crossbred cows in India: A place-based approach to rural development. Ann. Am. Assoc. Geogr. **99,** 746–766.

Brym, P., Kamiński, S. and Rusc, A. (2004): New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle. J. Appl. Genet. **45,** 445–452.

Coizet, B., Frattini, S., Nicoloso, L., Iannuzzi, L., Coletta, A., Talenti, A., Minozzi, G., Pagnacco, G. and Crepaldi, P. (2018): Polymorphism of the STAT5A, MTNR1A and TNFα genes and their effect on dairy production in *Bubalus bubalis*. Ital. J. Anim. Sci. **17,** 31–37.

Daldaban, F., Arslan, K., Akse, E. G. and Akyüz, B. (2020): Polymorphism of the STAT5A and MYF-5 genes in Anatolian water buffalo. Turk. J. Vet. Anim. Sci. **44,** 284–289.

Dario, C. and Selvaggi, M. (2011): Study on the STAT5A/AvaI polymorphism in Jersey cows and association with milk production traits. Mol. Biol. Rep. **38,** 5387–5392.

Dario, C., Dario, M., Ciotola, F., Peretti, V., Carnicella, D. and Selvaggi, M. (2009): Analysis of STAT5A/AvaI gene polymorphism in four Italian cattle breeds. Biochem. Genet. **47,** 671–679.

FAO (2006): The State of the World's Animal Genetic Resources for Food and Agriculture – First Draft, Rome. (http://www.pastoralpeoples.org/wp-content/uploads/2011/11/sowangr_sec1c.pdf).

Faria, F. J. C., Filho, A. E. V., Madalena, F. E. and Josahkian, L. A. (2009): Pedigree analysis in the Brazilian Zebu breeds. J. Anim. Breed Genet. **126,** 148–153.

Flisikowski, K., Hiendleder, S. and Zwierzchowski, L. (2005): Nucleotide sequence variation in the transcription factor STAT5A gene 5'-noncoding region in *Bos taurus* and *Bos indicus* cattle. Biochem. Genet. **43,** 459–464.

Flisikowski, K., Oprzadek, J., Dymnicki, E. and Zwierzchowski, L. (2003): New polymorphism in the bovine STAT5A gene and its association with meat production traits in beef cattle. Anim. Sci. Pap. Rep. **21,** 147–157.

Flisikowski, K., Strzałkowska, N., Słoniewski, K., Krzyżewki, J. and Zwierzchowski, L. (2004): Association of a sequence nucleotide polymorphism in exon 16 of the STAT5A gene with milk production traits in Polish Black-and-White (Polish Friesian) cows. Anim. Sci. Pap. Rep. **22,** 515–522.

He, X., Chu, M. X., Qiao, L., He, J. N., Wang, P. Q., Feng, T., Di, R., Cao, G. L., Fang, L. and An, Y. F. (2012): Polymorphisms of STAT5A gene and their association with milk production traits in Holstein cows. Mol. Biol. Rep. **39,** 2901–2907.

Hummeda, I., Alam, S., Makkawi, A., Sid-Ahmed, S., Abdoon, A., Kandil, O. and Hassanane, M. (2014): Restriction fragment length polymorphism for the Stat5a and Fgf2 genes in three Sudanese Zebu Cattle. GJASR **3,** 280–286.

Kmiec, M., Kowalewska-Luczak, I., Wojdak-Maksymiec, K., Kulig, H. and Grzelak, T. (2010): STAT5A/AvaI restriction polymorphism in cows of Polish Red-and-White variety of Holstein-Friesian breed. Russ. J. Genet. **46,** 81–85.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018): MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. **35,** 1547–1549.

Littlewood, R. W. (1936): Livestock of Southern India. Government Press, Madras, India. pp. 43–48.

Madalena, F. E., Peixoto, M. G. C. D. and Gibson, J. (2012): Dairy cattle genetics and its applications in Brazil. Livestock Res. Rural Dev. **24,** 97.

Manomohan, V., Saravanan, R., Pichler, R., Murali, N., Sivakumar, K., Sudhakar, K., Raja, K. N. and Kathiravan, P. (2021): Legacy of draught cattle breeds of South India: Insights into population structure, genetic admixture and maternal origin. PLoS One **16,** e0246497.

Miller, S. A., Dykes, D. D. and Polesky, H. F. (1988): A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. **16,** 1215.

Min, J. I., Liu, X. H., Chang-Lin, Y. U., Xuan-Fu, Y. U., Liu, D. J. and Shi, X. W. (2013): Association of STAT5A polymorphism and milk production traits in Binlangjiang buffalo. China Cattle Science **39,** 29–35.

Ogorevc, J., Kunej, T., Razpet, A. and Dovc, P. (2009): Database of cattle candidate genes and genetic markers for milk production and mastitis. Anim. Genet. **40,** 832–851.

Paramitasari, K. A., Sumantri, C. and Jakaria (2015): The genetic variability of prolactin and signal transducers and activators of transcription 5A (STAT5A) genes in Bali cattle. Media Peternakan **38,** 1–11.

Pellegrini, S. and Dusanter-Fourt, I. (1997): The structure, regulation and function of the Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). Eur. J. Biochem. **248,** 615–633.

Report (2017): Basic Animal Husbandry Statistics. Government of India, Ministry of Agriculture, Department of Animal Husbandry, Dairying And Fisheries, Krishi Bhawan, New Delhi.

Sadeghi, M., Moradi Shahrbabak, M., Rahimi Mianji, G. and Nejati Javaremi, A. (2009): Polymorphism at locus of STAT5A and its association with breeding values of milk production traits in Iranian Holstein bulls. Livestock Sci. **123,** 97–100.

Sanders, J. O. (1980): History and development of Zebu cattle in the United States. J. Anim. Sci. **50,** 118–120.

Selvaggi, M., Dario, C., Normanno, G., Celano, G. V. and Dario, M. (2009): Genetic polymorphism of *STAT5A* protein: relationships with production traits and milk composition in Italian Brown cattle. J. Dairy Res. **76,** 441–445.

Shortt, J. (1889): A Manual of Indian Cattle and Sheep: Their Breeds, Management and Diseases. Higginbotham & Co., Madras, India. p. 12.

Sutarno, S. and Setyawan, A. D. (2016): The diversity of local cattle in Indonesia and the efforts to develop superior indigenous cattle breeds. Biodiversitas **16,** 275–295.

Wakao, H., Gouilleux, F. and Groner, B. (1994): Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO J. **13,** 2182–2191.

Yeh, F., Yang, R. and Boyle, T. (2006): POPGENE Version 1.32. Microsoft Window Based Freeware for Population Genetic Analysis. University of Alberta and Center for International Forestry Research, Canada.