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Genetic diversity analysis of the thyroglobulin gene promoter in buffalo and other bovines

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ABSTRACT

In total 937 bp of the thyroglobulin (TG) gene, including promoter, exon1 and partial intron1 were characterized and compared across four livestock species, cattle, buffalo, yak and mithun. Identity was more than 98% and transcription factor binding sites analysis revealed the presence of variable numbers of potential binding sites in cattle, riverine buffalo, swamp buffalo, yak and mithun. The putative TTF-1 binding sites appeared to be conserved across all the investigated species except for a single C > T variation observed in *TG* promoter of Indicus cattle. A total of 15 polymorphic sites were observed in cattle and 8 in buffalo, out of which 2 were already reported in cattle and 4 polymorphic sites were common among cattle and buffaloes. The Principal component analysis results based on identified SNPs, revealed a close relationship between Crossbred and Indian Tharparkar breed of cattle as well as between Murrah and Banni breeds of buffalo. Analysis of the previously reported SNP, *Psu*I (g.−422C > T) marker (378C > T in our studies), associated with low marbling trait of meat in cattle, revealed an average frequency of 95% for the favourable C allele in Indian cattle, while it was fixed in the Indian swamp and riverine buffalo breeds. The study indicates, identification of genetic variation in the promoter region that could potentially affect the *TG* expression however, the association of the T allele with the fat deposition in Indian cattle and buffalo breeds needs to be verified.

1. Introduction

Buffalo and cattle are important livestock species which play an important role in milk and meat production in India. The Indian buffaloes comprise 56.7% of the world buffalo population, whereas the cattle population is 199.1 million, ranking second (15%) in the world (http://www.buffalopedia.cirb.res.in/). Combined cattle and buffalo contribute 90% percent to the total milk production in India and 60% to the meat production (http://www.fao.org/docrep/ARTICLE/AGRIPPA/665_en-01.htm). India ranks fourth in the world in the export of buffalo meat, which is about 2.15 million tons annually (http://en.mercopress.com/2012). The share of Indian meat exports in the world market is less than 2%. However, there is potential for augmentation, as this sector is largely unexploited.

Fat percentage is an important parameter for assessing the quality of milk and meat and it varies across species as

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well as across breeds within a species (Smet, 2012). Marbling and tenderness are two important traits of beef industry, which increase the carcass value by increasing its intramuscular fat, juiciness and flavor (Killinger et al., 2004). This diversity in fat percentage across species and breeds may be due to variations in the nucleotide sequence of the genes associated with fat metabolism. A QTL has been identified in the centromeric region of bovine chromosome 14 (BTA14), associated with the fat deposition in several cattle breeds (Casas et al., 2000; Moore et al., 2003). Genes lying in this region include Diacylglycerol-*O*-acyltransferase (*DGAT1*), thyroglobulin (*TG*) and adipose fatty acid binding protein (*FABP4*), that have been associated with fat percentage in both beef and dairy cattle (Barendse, 1999; Michal et al., 2006).

Thyroglobulin, is a glycoprotein hormone stored in the thyroid gland, that is synthesized in thyroid follicular cells and is a precursor of triiodothyronine (T3) and thyroxine (T4), playing an important role in regulating the metabolism and can affect adipocyte growth, differentiation and homeostasis of fat depots (Darimont et al., 1993). Polymorphism in *TG* has been associated with back fat thickness and marbling in beef cattle as well as milk traits in dairy cattle (Eenennaam et al., 2007; Hou et al., 2011). Subcutaneous fat thickness and fat percentage of tissues in general, including milk, are probably influenced by *TG* polymorphism as thyroid hormone levels influence milk fat percentage (Folley and Malpress, 1948). Diagnostic kits for testing *TG* polymorphisms associated with marbling and fat deposition are commercially available in cattle (GeneSTAR; Barendse, 1999; Barendse et al., 2004; Rincker et al., 2006).

The *TG* promoter region has been sequenced and characterized in several species including human (Christophe et al., 1985), bovine (Martynoff et al., 1987), canine (Donda et al., 1991), feline (Blackwood et al., 2001) and rat (Musti et al., 1987). Further, there are three thyroid transcription factor (TTF-1) binding regions A–C close to CAP site in the promoter region and any mutation in these sites might affect the expression of the *TG* gene (Javaux et al., 1992). The polymorphism g.-422C > T-(*Psu*I-RFLP) in *TG* promoter region has been associated with higher marbling scores in cattle with the homozygous T allele being favorable (Barendse, 1999; Thaller et al., 2003). This genetic variation occurring in the promoter region of the *TG* gene has been widely used in marker assisted selection programs to improve the predictability of marbling level and juiciness in beef cattle through a commercial DNA test. Information regarding the existence of similar/novel polymorphism in *TG* promoter region of Indian cattle and buffalo is completely lacking. Although milk fat percentage varies greatly between cattle and buffalo, significant variations have also been reported within species. Contrastingly, buffalo meat is reported to have less intra muscular fat with 1–2% marbling as compared to 3–4% in beef (Kandeepan et al., 2009) and is considered healthier. Hence, identification and validation of genetic variation for marbling in cattle and buffalo will assist in selective breeding to meet consumer demands of meat quality. The present study therefore was taken up to explore the diversity among the buffalo and other bovine species, known to be associated with marbling and tenderness, in the promoter region of the *TG* gene.

2. Material and methods

Three DNA samples each from riverine buffalo (*Bubalus bubalis*), swamp buffalo (*Bubalus bubalis carabanesis*), cattle

Table 1
Genotype and allelic frequencies of P*su*I genotype of thyroglobulin gene across different Indian cattle (*Bos taurus* and *Bos indicus*) and buffalo (riverine and swamp) populations.

Animal	No.	Genotype frequency			Allele frequency		Utility	Region
		CC	CT	TT	C	T		
Karan Fries	65	0.94	0.06	0.00	0.97	0.03	Dairy	HR
Mewati	26	0.88	0.08	0.04	0.92	0.08	Dual	RAJ. UP, HR
Hariana	20	0.95	0.05	0.00	0.98	0.03	Dual	HR
Gaolao	19	0.95	0.05	0.00	0.97	0.03	Dual	MH, MP
Kankrej	20	0.75	0.20	0.05	0.85	0.15	Dairy	GU, RAJ
Tharparker	20	0.85	0.15	0.00	0.93	0.08	Dairy	GU, RAJ
Gir	15	1.00	0.00	0.00	1.00	0.00	Dairy	GU
Rathi	20	1.00	0.00	0.00	1.00	0.00	Dairy	RAJ
Khillar	20	1.00	0.00	0.00	1.00	0.00	Draft	MH, KA
Amirtmahal	20	0.90	0.10	0.00	0.95	0.05	Draft	KA, SI
Nagori	21	0.48	0.52	0.00	0.74	0.26	Draft	RAJ
Hill cattle	10	0.90	0.10	0.00	0.95	0.05	Dairy	UK, HM
Holstein-Friesian	5	1.00	0.00	0.00	1.00	0.00	Dairy	HR
Cross Breed	4	0.50	0.50	0.00	0.75	0.25	Dairy	HR
Sahiwal	50	1.00	0.00	0.00	1.00	0.00	Dairy	PU, RAJ
Total	**335**	**0.90**	**0.09**	**0.01**	**0.95**	**0.05**	**Cattle**	
Swamp Buffalo	35	1.00	0.00	0.00	1.00	0.00	Meat/Draft	
Riverine Buffalo	38	1.00	0.00	0.00	1.00	0.00	Dairy	
Total	**73**	**1.00**	**0.00**	**0.00**	**1.00**	**0.00**	**Swamp/Riverine**	

*HR-Haryana, RAJ-Rajasthan, KA-Karnataka, SI-South India, MH-Maharashtra, UP-Uttar Pradesh, MP-Madhya Pradesh, PU-Punjab, HM-Himachal Pradesh, UK-Uttarakhand and GU-Gujarat.

(*Bos indicus*), mithun (*Bos frontalis*) and yak (*Bos grunniens*) were used for the amplification and sequencing of promoter, exon1 and partial intron1 region covering 937 bp of the *TG* gene.

For the detection of polymorphism in 548 bp of *TG* promoter region a panel of 42 unrelated animals belonging to five breeds of Indian buffalo and four breeds of Indian cattle were utilized. The buffalo breeds included were Murrah (3), Toda (3), Chilika (5), Banni (5) and Bhadawari (5), whereas the cattle breeds included crossbred Karan-Fries (Holstein friesian x Tharparkar, 6), Red Kandhari (4), Tharparkar (6) and Holstein-Friesian (5).

The *Psu*I-RFLP genotyping was carried out for the 378g.-C > T polymorphic site in our study (same as g.−422C > T SNP, reported), across 335 samples belonging to 15 cattle and 73 buffaloes (Table 1), belonging to various Indian riverine and swamp buffalo breeds/population.

2.1. PCR amplification

Total 937 nucleotides of *TG* gene including 667 nucleotides promoter and 270 nucleotides exon1 as well as part of intron1 was amplified in overlapping fragments using two sets of primers, P1 (Forward 5′-GGGGATGACTACGAGTATGACTG-3′ and Reverse 5′-GTGAAAATCTTGTGGAGGCTGTA-3′) reported by Barendse et al. (2004) and P2 (Forward 5′-TTAATGGATCTGCCTGTTTTGTTC-3′ and Reverse 5′-TCTAGTTTCCCATCTCCTGTCCAC-3′) designed by using PrimerSelect program of Lasergene software (DNASTAR Inc., Madison, WI, USA).

Primers set P1 (548 bp) was also used to amplify and screen polymorphism across the panel of 21 samples of each Indian cattle and buffalo breed, housing the reported *Psu1*-RFLP site (g.-378C > T), as well as for the genotyping of cattle and buffalo breeds (Table 1). Amplification was performed using programmable thermal cycler (PTC-200, MJ Research, USA) with an initial denaturation at 95 °C for 2.5 min followed by 35 cycles of 94 °C for 30 s, annealing temperature 58 °C for 30 s and 72 °C for 1 min, with a final extension of 5 min at 72 °C. The PCR products were visualized on 2% ethidium bromide stained agarose gel.

2.2. Sequencing

The amplicons were purified by enzymatic method using ExonucleaseI and Antarctic Phosphatase (New England Biolabs, USA). Purified PCR products were sequenced bi-directionally using BigDye terminator cycle sequencing kit (Applied Biosystems, USA) on ABI 3100 Genetic Analyzer and the raw sequence data was edited manually using Chromas Ver. 1.45 (http://www.technelysium.com.au/chromas.html).

2.3. PsuI-RFLP (g.-378C > T) genotyping

The PCR amplified products from primers P1 (548 bp) were digested with *Psu*I restriction enzyme (New England BioLabs) in a 20 μl reaction volume at 37 °C for 8 h. The RE digested products were electrophoresed in 3% agarose gel and the genotypic patterns were visualized by ethidium bromide staining and recorded for further analysis.

2.4. Data analysis

Two different fragments of *Bubalus bubalis* (riverine buffalo), *Bubalus bubalis carabanesis* (swamp buffalo), *Bos indicus* (cattle), *Bos frontalis* (mithun) and *Bos grunniens* (yak) of *TG* gene were assemble into 937 bp using seqMan program of Lasegene software, analysed and submitted to GenBank (Acc. no. JX09179-JX09183). These sequences were compared with published bovine sequence (ENSBTAT00000010295). Transcription factor binding site analysis was performed using Alibaba and P-Match gene regulation online programme (http://www.gene-regulation.com/pub/programs.html).

For detection of polymorphism the edited sequences were further subjected to multiple alignments to identify nucleotide variations, using Mega4 program. Allele frequency for each breed and F_{ST} value (Weir and Cockerham, 1984) were calculated using GenAlex6.2 program (Peakall and Smouse 2006). A principal component analysis (PCA) was carried out to determine breed relationships among buffalo and cattle directly based on F_{st} values (Manly, 1986), using GenAlEx6.2 program.

3. Results

3.1. Diversity across species

Promoter and adjoining exon 1 and part of intron 1 sequence data amplified as 937 bp product, when analyzed, there was 99% homology between Zebu and Taurus *TG* promoter sequence. Among the species/subspecies investigated, maximum homology (99.8%) was between riverine and swamp buffaloes. Overall 98% homology was observed across the four species. Phylogenetic analysis showed grouping of two buffalo subspecies into separate cluster whereas, all the three *Bos* species, cattle, yak and mithun clustering together in another group (Fig. 1). Transcription factors binding sites analysis revealed the presence of 59 potential sites in cattle, mithun and riverine buffalo, 58 in swamp buffalo and 60 in yak. Most of the transcription factor binding sites were conserved across species, while some sites exhibited variation between species (Fig. 2). The three putative thyroid transcription factor (TTF-1) binding sites A, B and C reported in other species (Donda et al., 1991) were largely conserved across the species investigated. However in the C region, a C > T

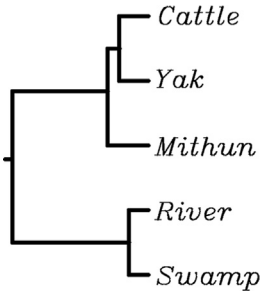

Fig. 1. Phylogenetic relationship between cattle, yak, mithun and buffalo (riverine and swamp), based on nucleotides sequence of thyroglobulin gene promoter.

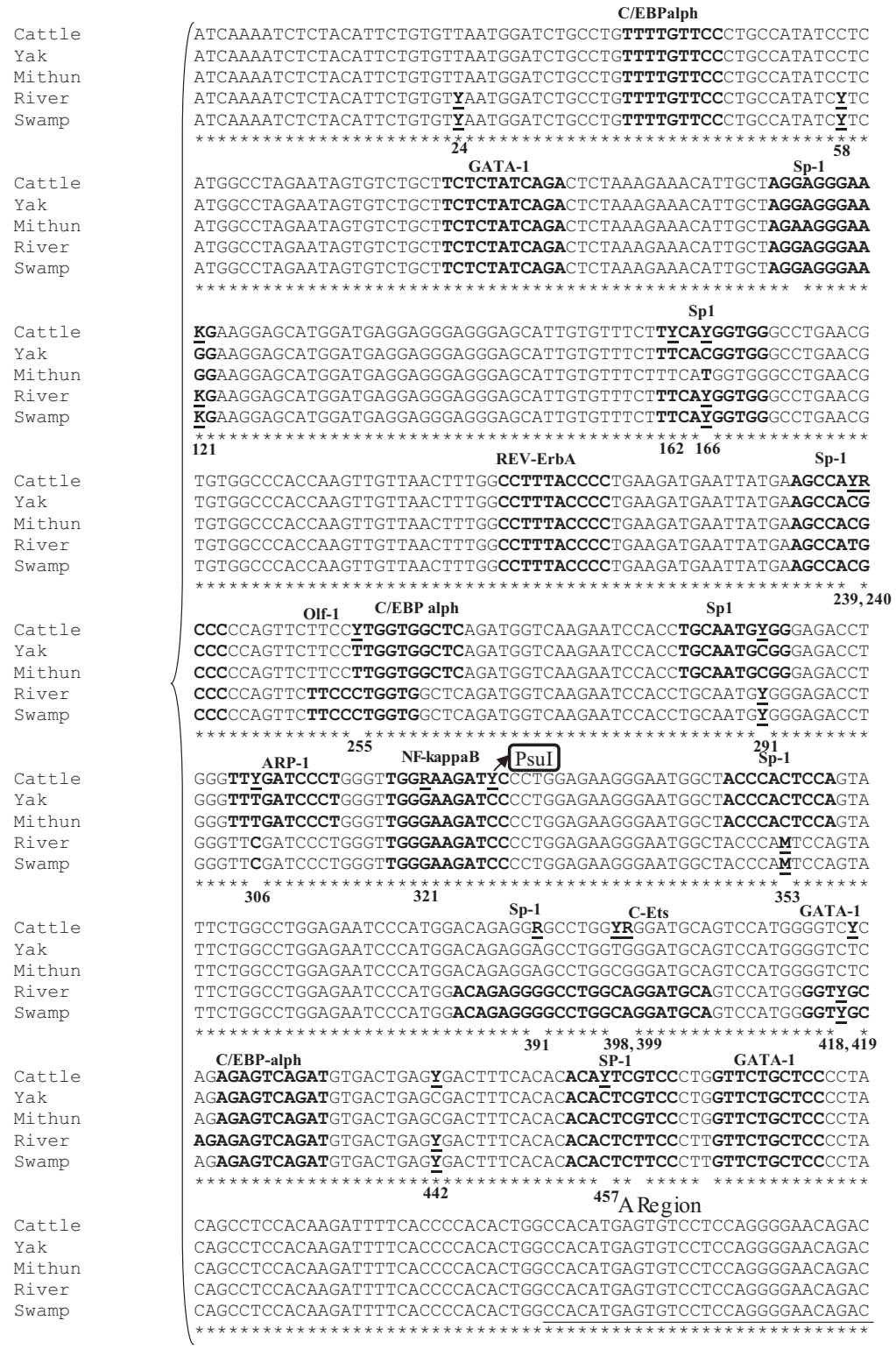

Fig. 2. Alignment of thyroglobulin promoter sequences of cattle (Acc. no. JX090179), Mithun (Acc. no. JX090180), yak (Acc. no. JX090183), riverine (Acc. no. JX090181) and swamp (Acc. no. JX090182) buffaloes showing variation in putative transcription binding factors binding sites and polymorphism with their respective positions as highlighted ones.

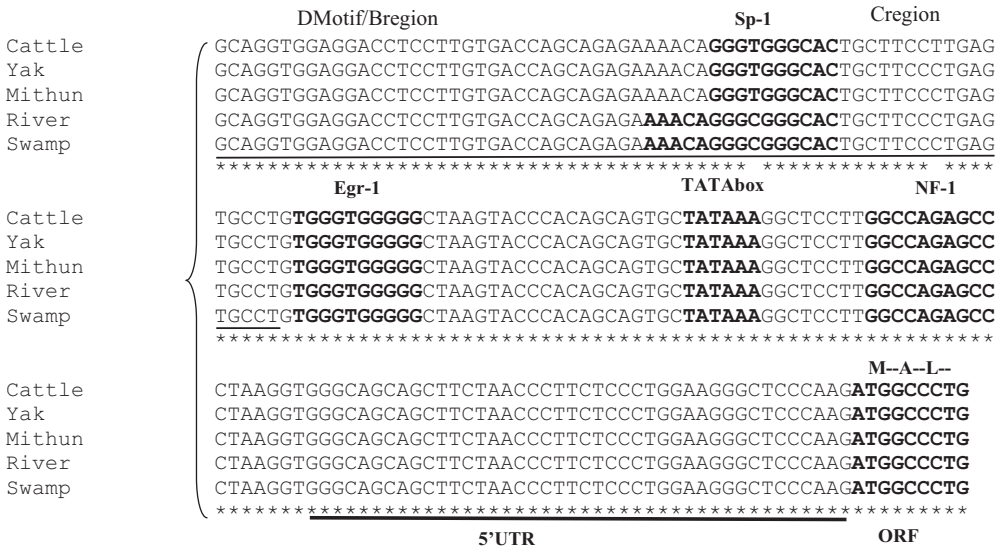

Fig. 2. (*continued*)

transversion was detected at position 596 in Indian cattle (Fig. 2).

3.2. Polymorphism across Indian cattle and buffalo

The sequence analysis of the 548 nucleotides region, reported to be harboring polymorphic sites associated with marbling in feedlot cattle (Barendse et al., 2004), revealed a total of 15 polymorphic sites in cattle and 8 in buffalo (Tables 2a and 2b). Of the 15 SNPs identified in Indian cattle two SNPs, 291A > G and 378C > T (g.-422C > T) have been previously described in Korean and other cattle (Shin and Chung, 2007), rests thirteen being novel. Four polymorphic sites were common across cattle and buffalo (121G > T, 166C > T, 291C > T and 442C > T). The allele and genotype frequencies for each of the identified SNPs were estimated across cattle and buffalo breeds as reported by earlier workers in candidate gene polymorphism across breeds/populations (Yoon et al., 2005; Hou et al., 2011; Sodhi et al., 2013). The frequency of C allele at position 378, reported to be associated with marbling, was 95% in cattle and completely fixed in buffalo (Tables 2a and 2b). Based on these SNPs, the relationship between the cattle as well as buffalo breeds was analyzed using principal component analysis of GenAlEx 6.2 program. The results revealed a close relationship between Tharparkar and crossbred cattle Karan-Fries (Cross of taurine Holstein and indicus Tharparkar breed) as compared to Red Kandhari and Holstein Friesian breeds of cattle. In case of buffalo Murrah and Banni breeds showed a greater genetic similarity (Fig. 3).

3.3. Analysis of −*Psu*I (SNP g.−378C > T) genotypes in Indian cattle and buffalo

Allelic distribution of *Psu*I (378C > T) polymorphism observed in the promoter region of the thyroglobulin gene, associated with meat tenderness in cattle was analyzed across 335 animals, including 15 documented Indian cattle breeds of different agro- climatic regions and of different utility, as well as 73 samples of Indian riverine (38 animals) and swamp buffaloes (35 animals). The distribution of genotypes and allele frequencies for all the breeds of cattle and buffalo obtained by PCR-RFLP analysis is summarized in Table 1. In indigenous cattle and buffalo the −*Psu*I RFLP revealed a banding pattern similar to that reported previously (Thaller et al., 2003). The C allele yielded three fragments of 295, 178 and 75 bp, while the T allele gave two fragments of 473 and 75 bp sizes, respectively. The average allelic frequency for alleles C and T was 0.95 and 0.05, respectively, across all the cattle breeds investigated (Table 1). However the C allele was fixed in Indian buffaloes. The distribution of both alleles was similar across all the indigenous cattle breeds irrespective of their geographic distribution and utility. However, among indigenous breeds, frequency of T allele was comparatively higher in Kankarej (0.15) and Nagori (0.26), mostly due to presence of CT heterozygotes. The homozygous TT genotype was absent in all the animals except in Mewati and Kankrej cattle breeds. The frequency of the CC genotype was higher than CT in all cattle breeds except Nagori.

4. Discussion

4.1. Diversity in TG promoter region across species

The thyroglobulin promoter alongwith exon1 and partial intron1 region, was sequence characterized in four species. Overall homology above 98% was observed across all the species studied. The genetic relationship of yak (*B. grunniens*) was closer to cattle (*Bos taurus*) than mithun (*B. frontalis*) (Fig. 1), which appeared to be similar to previous reports based on microsatellite/mitochondrial diversity studies (Dorji et al., 2010; Qi et al., 2009). Since the expression of genes is largely regulated by binding of

Table 2a
Genotype and allelic frequencies of SNPs observed in thyroglobulin gene of Indian riverine buffaloes.

Position*	No.	Genotype frequencies			Allele frequencies	
B24 C > T	21	0.29 (CC)	0.29 (CT)	0.43 (TT)	0.43 (C)	0.57(T)
B58 C > T	21	0.90 (CC)	0.05 (CT)	0.05 (TT)	0.93 (C)	0.07 (T)
B121G > T	21	0.29 (GG)	0.43 (GT)	0.29 (TT)	0.5 (G)	0.5 (T)
B166 C > T	21	0.33 (CC)	0.38 (CT)	0.29 (TT)	0.52 (C)	0.48 (T)
B291C > T	21	0.43 (CC)	0.33 (CT)	0.24 (TT)	0.6 (C)	0.4 (T)
B353 A > C	21	0.24 (AA)	0.33(AC)	0.43 (CC)	0.4 (A)	0.6 (C)
B418 C > T	21	0.43 (CC)	0.33 (CT)	0.24 (TT)	0.6 (C)	0.4 (T)
B442 C > T	21	0.95 (CC)	0.05 (CT)	0.0(TT)	0.98 (C)	0.02 (T)

*Position with respective buffalo Acc. no. JX090181.

Table 2b
Genotype and allelic frequencies of SNPs observed in thyroglobulin gene of Indian cattle.

Position*	No.	Genotype frequencies			Allele frequencies	
C121 G > T	21	0.43(GG)	0.57 (GT)	0.00 (TT)	0.71(G)	0.29 (T)
C162 C > T	21	0.24 (CC)	0.57(CT)	0.19 (TT)	0.52 (C)	0.48 (T)
C166 C > T	21	0.33 (CC)	0.38(CT)	0.29 (TT)	0.52 (C)	0.48 (T)
C239 C > T	21	0.43 (CC)	0.57(CT)	0.00 (TT)	0.71 (C)	0.29 (T)
C240 A > G	21	0.24 (AA)	0.57 (AG)	0.19 (GG)	0.52 (A)	0.48 (G)
C255 C > T	21	0.00 (CC)	0.57(CT)	0.43 (TT)	0.29 (C)	0.71 (T)
[a]C291 C > T	21	0.90 (CC)	0.05(CT)	0.05 (TT)	0.93 (C)	0.07 (T)
C306 C > T	21	0.00 (CC)	0.57(CT)	0.43 (TT)	0.29 (C)	0.71 (T)
C321 A > G	21	0.05 (AA)	0.05(AG)	0.90 (GG)	0.07 (A)	0.93 (G)
[a]C378 C > T	335	0.90 (CC)	0.09(CT)	0.01 (TT)	0.95 (C)	0.05 (T)
C391 A > G	21	0.43 (AA)	0.57(AG)	0.00 (GG)	0.71 (A)	0.29 (G)
C398 C > T	21	0.90 (CC)	0.10(CT)	0.00 (TT)	0.95 (C)	0.05 (T)
C399 A > G	21	0.00 (AA)	0.57(AG)	0.43 (GG)	0.29 (A)	0.71 (G)
C419 G > T	21	0.00 (GG)	0.57 (GT)	0.43 (TT)	0.29 (G)	0.71 (T)
C442 C > T	21	0.90 (CC)	0.05(CT)	0.05 (TT)	0.93 (C)	0.07 (T)
C457 C > T	21	0.19 (CC)	0.57(CT)	0.24 (TT)	0.48 (C)	0.52 (T)

[a] SNPs previously reported were observed in Indian cattle, Position with respective to cattle Acc. no. JX090179.

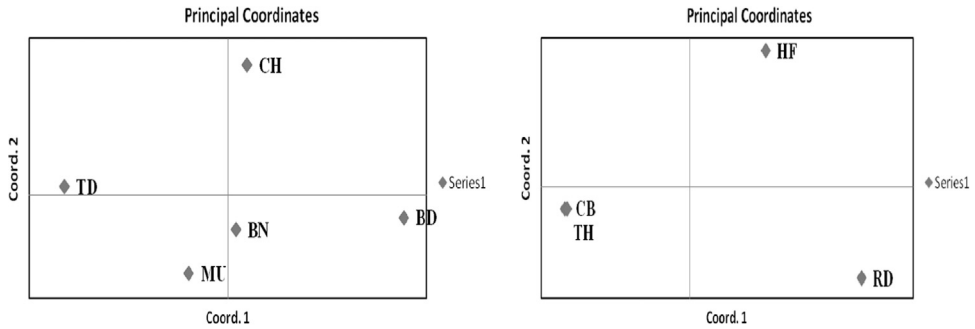

Fig. 3. Principal component analysis of different breeds of buffalo (MU-Murrah, BN-Banni, BD-Bhadawari, TD-Toda and CH-Chilika) and cattle (*Bos indicus* and *Bos taurus*: CB-Crossbred, TH-Tharparkar, RD-Red Kandhari and HF-Holstein Friesian) on the basis of F_{st} values.

transcription factors in the promoter region, changes in the transcription factors binding sites might affect its expression. In the present study the putative TTF-1 (A–C regions) and TATA binding site were conserved across the investigated species. Although in the rat the three TTF-1 sites are much more conserved in terms of DNA sequences, they display better conservation of function in the cow (Javaux et al., 1992). In human, Fabbro et al. (1994) found that, in anaplastic thyroid carcinomas, the absence of TTF-1 was associated with the absence of *TG* expression. Mutations in the transcription factor binding sites have been reported to alter/affect the expression of many genes such as amyloid precursor protein (*APP*) gene in humans (Theuns et al., 2006), ankyrin gene in bovine (Aslan et al., 2010) and *IGF2* gene in pigs (Aslan et al., 2012). Therefore, the significance of the 596C > T variation observed in the TTF-1 binding C region of the *TG* promoter in Indian cattle needs to be further investigated.

4.2. Polymorphism in cattle and buffalo TG promoter

In this study, 13 novel and 2 previously reported polymorphic nucleotide sites could be detected in 548 bp of the *TG* gene promoter region of cattle and 7 of the SNPs

detected were novel in buffalo. The presence of new polymorphisms at this region reflects a greater genetic diversity available in Indian cattle and buffalo populations at this locus. This genetic polymorphism may account for the between species and within species variation in the milk fat content of cattle and buffalo (Medhammar et al., 2011).

4.3. Analysis of PsuI (SNP g.−378C > T) genotypes

Analysis of the g.−378C > T (*Psu*I) SNP revealed that all cattle breeds as well as buffalo, irrespective of their geographic distribution and utility exhibited clear predominance of C allele. These results are in agreement with previous reports on *B. taurus* and *B. indicus* cattle (Eenennaam et al., 2007; Fortes et al., 2009). The C allele was fixed in Gir, Rathi, Khillar and Sahiwal breeds and except for Khillar these are dairy breeds, having moderately higher percentage of fat in milk as compared to taurine cattle. The buffalo samples also showed fixation of the C allele. It is worth emphasizing that the homozygous TT genotypes were largely absent in the Indian cattle and totally absent in buffalo populations. Several studies have associated this polymorphism with marbling, intramuscular fat, backfat thickness, ribeye area and carcass weight (Barendse, 1999; Thaller et al., 2003; Casas et al., 2005; Rincker et al., 2006; Shin and Chung, 2007). The g.−378C > T polymorphism, was established as the causative mutation within the marbling QTL (Barendse et al., 2004) and the T allele was considered the favorable allele for intramuscular fat deposition. This polymorphism has since been used in commercial DNA marker kits (*TG*5 marker, GENESTAR). It is interesting to note that Indian cattle breeds like Kankrej, and Nagori exhibited a slightly higher frequency of the T allele (0.26), comparable to that observed in Angus and Shorthorn breeds (Eenennaam et al., 2007). Higher frequency of T allele in Nagori was due to presence of CT genotypes despite that TT genotypes were missing, which could be due to recent decline in its population being a draft breed and introgression of other breeds. Contradictory reports are also available, where no association was found between the *TG* polymorphism and back fat in *B. taurus* (Moore et al., 2003) or marbling score in *B. indicus* cattle (Casas et al., 2005). Pannier et al. (2010) also did not find any association with marbling and fat deposition traits in various cattle breeds. A lower frequency of the T allele in *B. indicus* as compared to *B. taurus* has also been observed previously (Eenennaam et al., 2007; Fortes et al., 2009; Carvalho et al., 2012). Shin and Chung (2007) have reported the association of CC and CT genotypes with a higher marbling score in Korean cattle. Interestingly, we found this polymorphism within NF-κB transcription factor binding site that governs the cellular reaction to a variety of extracellular signals (Fig. 2) (Diamant and Dikstein, 2013). Since the effects of these genotypes may vary among different populations and with environment, the association of the T allele with fat deposition in Indian cattle and buffalo needs to be verified, however, its absence or low frequency suggests results to be similar to reported in Korean cattle, since milk fat contents of Indian cattle as well as buffalo is higher than taurine cattle. The diversity available in Indian cattle breeds for g.−378C > T SNP, as revealed from this study, also presents the opportunity to exploit the use of either allele/genotype once the association is validated in the Indian bovine and bubaline population. In order to confirm these observations future studies will need to be carried out to see the effect of reported SNPs on the milk and meat quality traits, in the Indian cattle and bubaline populations.

The elucidation of genetic polymorphism in the promoter region of *TG* gene in five species as well as analysis of the g.−378C > T polymorphism in Indian cattle and buffalo has been presented in the study. The low frequency of the T allele in Indian breeds was in conformity to previous reports in *B. indicus* (Fortes et al., 2009; Carvalho et al., 2012). However, an in depth analysis of the association of the *TG* marker in Indian cattle and buffalo is required in order to utilize it for improvement of the meat/milk quality.

Conflict of interest statement

None.

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