https://doi.org/10.17221/206/2021-CJAS

Molecular characterization and *A1/A2* genotyping of casein beta gene in zebu and crossbred cattle of Bangladesh

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Citation: Pabitra M.H., Maruf T.M., Mony S.I., Ebnat R., Hoque M.R., Bhuiyan M.S.A. (2022): Molecular characterization and *A1/A2* genotyping of casein beta gene in zebu and crossbred cattle of Bangladesh. Czech J. Anim. Sci., 67: 318–326.

Abstract: Casein beta (*CSN2*) is the most explored gene in cattle due to its potential impact on human health. Here, we investigated the entire coding sequence of *CSN2* gene except the last two codons for detection of polymorphisms in different cattle populations of Bangladesh as well as genotyping of *A1* and *A2* allelic variants using a newly developed allele specific polymerase chain reaction (AS-PCR) based the genotyping protocol. Five primer pairs were used to amplify the coding sequences of *CSN2* gene. This study included a total of 258 DNA samples from six Bangladeshi zebu populations and one Holstein Friesian (HF) derived crossbred cattle population. Sequence analysis detected five nonsynonymous mutations in the coding sequence of *CSN2* gene that defined five allelic variants as *A1, A2, B, F* and *I*. In addition, substitution of GTA (Val) by GCA (Ala) at position 197 resulted in an undefined allele in the zebu cattle population of Bangladesh that has not yet been reported elsewhere. Like other *Bos indicus* cattle populations, *A2* allele is predominant in the studied zebu populations. The mean frequencies of *A1A1, A1A2* and *A2A2* genotypes were 0.02, 0.16 and 0.82, respectively, in zebu cattle populations while the corresponding allele frequencies were *A1* (0.10) and *A2* (0.90). In opposite, the aforesaid genotype frequencies were 0.14 (*A1A1*), 0.50 (*A1A2*) and 0.36 (*A2A2*) in the HF crossbred population with allele frequencies of 0.39 (*A1*) and 0.61 (*A2*). The adopted AS-PCR method was found cost-effective, rapid and had high specificity for genotyping of *A1* and *A2* allelic variants. Altogether, this study provides information for the selection of desired zebu and crossbred individuals in order to produce premium quality milk as well as to design a breeding plan in the crossbreeding program.

Keywords: allelic variant; coding sequence; AS-PCR genotyping

Milk is a nature's perfect food which provides an important source of nutrients including proteins, lactose, minerals and vitamins essential for brain and body development. Milk protein is mainly of two types that is composed of about 80% casein and 14% whey protein (McLachlan 2001).

Supported by the Bangladesh Agricultural University Research System (BAURES), Mymensingh, Bangladesh (Project No. 2019/797/BAU).

https://doi.org/10.17221/206/2021-CJAS

There are four types of casein available in bovine milk such as casein alpha s1 (CSN1S1, 39–46%), casein alpha s2 (CSN1S2, 8–11%), casein beta (CSN2, 25–35%) and casein kappa (CSN3, 8–15%). In addition, casein gamma derives from the degradation of CSN2 (Kaminski et al. 2007). Milk caseins are encoded by four members of the casein gene family located on bovine chromosome 6 (Rijnkels 2002). *CSN2* is the second most abundant milk protein that consists of 209 amino acids (Hanusova et al. 2010). It plays important roles in cheese yield or quality and renneting properties of milk (Boettcher et al. 2004), and as a molecular marker for milk production traits in dairy cattle (Olenski et al. 2010). The CSN2 is the most polymorphic milk protein gene in cattle. Fifteen genetic variants of the *CSN2* gene (*A1, A2, A3, B, C, D, E, F, G, H1, H2, I, J, K,* and *L*) have been identified, which are responsible for substitution of certain amino acids in the CSN2 protein and alter its properties (Dai et al. 2016; Meier et al. 2019). *A1* and *A2* are the most common variants in dairy cattle breeds that differ in a single nucleotide change (CCT>CAT) leading to substitution of proline (*A2*) by histidine (*A1*) at position 67 in the amino acid sequence. The polymorphisms of *CSN2* gene are also responsible for conformational changes in the secondary structure of expressed CSN2 protein (Hanusova et al. 2010). This point mutation occurred thousands years ago in some European cattle breeds. The human enzymatic digestion results in cleavage between histidine and adjacent amino acid successively and thereby releases a 7-amino acid bearing a bioactive peptide 'opioid' called beta-casomorphin 7 (BCM-7) in the human small intestine. BCM-7 can potentially affect numerous opioid receptors in the cardiovascular, respiratory, nervous system and immune system in humans (Bell et al. 2006). More particularly, it has been reported that BCM-7 (A1 variant) is considered a risk factor for several human diseases and disorders like ischemic heart diseases, insulin-dependent diabetes, atherosclerosis, schizophrenia, autism and milk intolerance (Caroli et al. 2009; Mishra et al. 2009). In contrast, epidemiological evidence suggests that the original A2 casein beta variants (A2 milk) have no such health hazard and is better for human health than A1 milk (McLachlan 2001).

In respect to human health issues continuous awareness has been built in favour of A2 milk for the last several years. A2 branded milk is now available categorically as premium quality milk in the supermarket. Nowadays, genotyping for *A1* and *A2* casein beta variants has been practiced routinely in the sire selection program of every renowned breeding service provider worldwide (Meier et al. 2019). Earlier studies reported *A1* casein beta variants for the majority of the *Bos taurus* dairy breeds except Jersey and Guernsey those having *A2* variant (Kaminski et al. 2007). In contrast, *A2* variant is predominant in *Bos indicus* cattle breeds (Mishra et al. 2009; Kumar et al. 2019). However, there is no report on genetic characterization of the *CSN2* gene and its allelic frequencies (*A1* and *A2*) in the zebu and upgraded or crossbred populations of Bangladesh.

In Bangladesh, zebu cattle have several distinct varieties or types such as North Bengal Grey (NBG), Red Chittagong (RCC), Pabna (PC), Munshiganj (MC) and non-descript Deshi (DES), those are classified based on their coat colour, productivity and morphometric features (Hamid et al. 2017). Besides, good numbers of Sahiwal (SL) cattle are also discernible across the country. It is notable to mention that a considerable number of crossbreds are nowadays discernible across the country due to the upgradation program with high-yielding temperate and tropical dairy breeds for the last five decades (Bhuiyan et al. 2015) and their proportion is increasing day by day. Therefore, it would be worthwhile to know the allelic status of Bangladeshi cattle populations in order to select the desired genotypes for production of premium quality milk. Under the above stated circumstances, the objectives of this study were to characterize the entire coding sequence of *CSN2* gene in different cattle populations of Bangladesh as well as to investigate the *A1* and *A2* allelic patterns using newly developed allele specific genotyping.

MATERIAL AND METHODS

Ethical statement

Approval was taken from the Institutional Ethical Standard of Research Committee (IESRC) of Bangladesh Agricultural University (Approval number: ESRC/AH/09) before commencement of the study and IESRC guidelines were followed during experimentation.

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Sampling and DNA extraction

The genotypes of the animals were ascertained based on phenotypic features, pedigree records as well as interaction with farmers or responsible persons. Blood samples were collected randomly from different zebu cattle varieties of Bangladesh (RCC, PC, SL, NBG, MC, DES), and Holstein derived (HF) crossbreds. Samples were collected from unrelated yearling and adult individuals. This study includes 258 animals that comprised 133 HF crossbreds and 125 zebu individuals maintained at different institutional herds and farmers. About 2–3 ml of blood sample were collected using a vacutainer coated with EDTA as anticoagulant and stored at 4 °C until DNA extraction. Genomic DNA was extracted from whole blood using a PrimePrep Genomic DNA Extraction kit (Add Bio Inc., Daejeon, South Korea). Purity and concentration of the DNA sample were assessed by a spectrophotometer (Nanodrop 2000, Thermo Fisher Scientific, Waltham, MA, USA) and the extracted DNA samples were stored at –20 °C for further study.

PCR amplification and sequencing

A total of five primer pairs were used to amplify the coding sequences comprising exon 1 to 7 of *CSN2* gene. All allelic variants so far identified are located in these seven exons. In exon 8, there are only six coding nucleotides (two codons: for Val and stop), and these have not been included in the amplification process. The first three primer pairs were designed using NCBI Primer Blast software (www.ncbi.nlm.nih.gov/tools/primer-blast/) based on NCBI reference sequence NC_037333.1 (position: 85 449 173 to 85 457 867 bp, reverse complement) and the latter two pairs reported by Dai et al. (2016) were included in this study. The sequence information, fragment size, optimized annealing temperature are presented in Table 1. PCR reactions were carried out in a 20 µl volume containing 1× buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM each primer and 0.3 U *Taq* DNA polymerase (GeNet Bio Inc., Daejeon, South Korea) and approximately 50 ng genomic DNA. The thermal profile comprised initial denaturation at 95 °C for 10 min followed by 30 cycles with denaturation at 95 °C for 30 s, annealing at 59–63 °C for 30 s and extension at 72 °C for 1 min and final extension at 72 °C for 10 minutes. PCR products were visualized on 2.0% agarose gel stained with 5 µl (10 µg/µl) of safe dye (green gel) and the images were captured by GDS 200 digital gel documentation system (Sunil Bio Inc., Seoul, South Korea). PCR products were purified using a PrimePrep PCR purification kit (GeNet Bio Inc., Daejeon, South Korea). Purified PCR products of eight samples from each gene fragment were then sequenced both in forward and reverse directions from a commercial sequencing provider (Macrogen, Seoul, South Korea). Sequencing was performed by using automated Genetic Analyzer 3130xl (Applied Biosystems, Carlsbad, CA, USA).

A1/*A2* genotyping

Due to the absence of restriction site near the coding sequence for amino acid at position 67,

Table 1. Primer sequence information for amplification of exonic regions of *CSN2* gene

Primer name	Amplified region	Primer sequence	Amplicon size (bp)	Annealing temp (°C)
CSN2-E1F	exon 1	5'-TTTGGGGACAGAAAAATAGG-3'	410	59
CSN2-E1R		5'-CCTGTTAACTAGTCTCGTGG-3'		
CSN2-E2F	exon 2	5'-GATCTTGGTTCCCTGATGAG-3'	626	62
CSN2-E2R		5'-AGCACTCTACAGTAAGGGAT-3'		
CSN2-E3F	exon 3 and 4	5'-TCCCTTACTGTAGAGTGCTAGGT-3'	689	63
CSN2-E3R		5'-TCCTGTCCGACTCTTAGCGA-3'		
CSN2-E5F	exon 5 and 6	5'-CACCTGGGAAGCCCGATTAG-3'	723	62
CSN2-E5R		5'-AGGGGATCACAGTCCCTAGTC-3'		
CSN2-E7F	exon 7	5'-AGGCAACTCAGGAAGAGGTG-3'	995	63
CSN2-E7R		5'-ATCTCCACGGGTAAGCCTAGA-3'		

https://doi.org/10.17221/206/2021-CJAS

allele specific primers were designed to investigate the *A1* and *A2* allelic patterns in zebu and crossbred cattle populations of Bangladesh with a view to establish *A1* and *A2* genotyping. The primers were designed through inclusion of mismatched nucleotide at the 3' terminal end of the primers. The sequence information of allele specific primers is given in Table 2. The 20 µl PCR reaction volume contained 2 µl (~100 ng) DNA, 10 µl of 2x Master mix (*Taq* DNA polymerase 2 U, 20 mM Tris-HCl, 100 mM KCl, 0.2% Triton® X-100, 2 mM $MgCl_2$, and 0.5 mM each of dNTP) and 8 µl allele specific primer mixture (100 pmol of each primer). The primer mixture contained four different primers that were used together in one reaction. PCR amplification comprised initial denaturation at 95 °C for 10 min followed by 30 cycles with denaturation temperature at 95 °C for 20 s, annealing at 60 °C for 20 s; extension at 72 °C for 20 s and finally the reaction was completed with final extension at 72 °C for 5 minutes. Then the PCR products were loaded on 2% agarose gel containing 1x TBE buffer and the gel was visualized by GDS 200 digital gel documentation system to observe and record the results.

Sequence analysis

The generated raw sequences were edited and aligned using bioinformatic tools BioEdit 7.2 (Hall 1999); ClustalW (Larkin et al. 2007) and MEGA v7.0 (Kumar et al. 2016) for mutation scoring as well as to ascertain the sequence of the respective gene fragment. The resultant DNA fragments of this study were aligned to the bovine *CSN2* gene (GenBank accession No. NC_037333.1; position 85 449 173 to 85 457 867; reverse complement) to obtain the entire coding sequence (without two codons). Genotype and allele frequencies at *A1*/*A2* locus were calculated. The amino acid positions were determined according to the mature CSN2 protein reported by Bonsing et al. (1988).

RESULTS AND DISCUSSION

Detection of polymorphisms in *CSN2* gene

In the present study we detected a number of genetic variants in the coding sequences along with adjacent intronic regions of *CSN2* gene in zebu and crossbred cattle populations of Bangladesh and they are enlisted in Table 3. In total, five nonsynonymous mutations were found in the coding sequence of exon 7 which led to amino acid changes based on the reference sequence NC_037333.1 (position 85 449 173 to 85 457 867; reverse complement). The g.6570C>A (Pro to His) and g.6736C>G (Ser to Arg) mutations were predominantly available in both zebu and crossbred populations. Two other missense mutations g.6647A>C (Met to Leu) and g.6825C>T (Pro to Leu) were identified only in crossbred populations where the g.6960T>C (Val to Ala) SNP was found indicine specific [Figure S1 in electronic supplementary material (ESM); for the supplementary material see the electronic version]. However, three silent mutations were detected in exon 2 (g.2203C>T), exon 3 (g.5151G>A) and exon 7 (g.6730T>C) of *CSN2* gene. In addition, we found mutations in the intronic regions of *CSN2* gene of the sequenced samples, those were in intron 2 (g.2296C>T; g.2348T>G), intron 3 (g.3046A>G), intron 4 (g.3177G>T), intron 5 (g.5076A>T; g.5133G>A) and intron 6 (g.6491T>C). Milk proteins are a good source of bioactive peptides. *CSN2* is the most explored gene in the casein family due to its potential impact on human health (Kaminski et al. 2007). Caroli et al. (2009) stated a total of 12 genetic variants that comprise at least 13 amino acid exchanges of *CSN2* gene in different cattle breeds.

Sequence characterization of *CSN2* gene

Table 4 represents the identified casein beta variants and their respective positions corresponding

Table 2. Allele specific primer sequences information

Primer name	Primer sequence	Amplicon size (bp)
Common forward	5'-CCTTCTTTCCAGGATGAACTCCAG-3'	277
Common reverse	5'-CAACATCAGTGAGAGTCAGGCTC-3'	
AA-geno-forward	5'-CTTCCCTGGGCCCAACCA-3'	277, 209
GG-geno-reverse	5'-GATGTTTTGTGGGAGGCTGATAG-3'	277, 108

https://doi.org/10.17221/206/2021-CJAS

Table 3. Identified SNPs in the *CSN2* gene of zebu and crossbred cattle genotypes of Bangladesh

Location	SNP position[1]	Population	Amino acid substitution	Amino acid position[2]
Exon 2	g.2203C>T	zebu	synonymous	A-9A
Intron 2	g.2296C>T	crossbred	–	–
Intron 2	g.2348T>G	zebu and crossbred	–	–
Intron 3	g.3046A>G	zebu	–	–
Intron 4	g.3177G>T	zebu	–	–
Intron 5	g.5076A>T	crossbred	–	–
Intron 5	g.5133G>A	zebu	–	–
Exon 6	g.5151G>A	zebu and crossbred	synonymous	Q34Q
Intron 6	g.6491T>C	crossbred	–	–
Exon 7	g.6570C>A	zebu and crossbred	missense (Pro>His)	P67H
Exon 7	g.6647A>C	crossbred	missense (Met>Leu)	M93L
Exon 7	g.6730T>C	zebu	synonymous	T120T
Exon 7	g.6736C>G	zebu and crossbred	missense (Ser>Arg)	S122R
Exon 7	g.6825C>T	crossbred	missense (Pro>Leu)	P152L
Exon 7	g.6960T>C	zebu	missense (Val>Ala)	V197A

[1]SNP position is based on the *CSN2* reference sequence of *Bos taurus* (NC_037333.1; position: 85 449 173 to 85 457 867 bp, reverse complement)

[2]Numbering is relative to the beginning of mature protein (XP_010804480.2; the beginning of the mature peptide in this sequence is at position 51)

Table 4. Polymorphic nucleotides and corresponding amino acid changes in five casein beta variants observed among the investigated cattle of Bangladesh

CSN2 variants	Position and amino acid in the CSN2 protein*				
	67	93	122	152	197
A2	Pro (C**C**T)	Met (**A**TG)	Ser (AG**C**)	Pro (C**C**T)	Val (G**T**A)
A1	His[1,2] (C**A**T)	–	–	–	–
B	His (C**A**T)	–	Arg[2] (AG**G**)	–	–
F	His (C**A**T)	–	–	Leu[2] (C**T**T)	–
I	–	Leu[2] (**C**TG)	–	–	–
Not yet defined	–	–	–	–	Ala[3] (G**C**A)

*The reference protein sequence is based on XP_010804480.2; mature peptide (from amino acid 51) corresponding to the CSN2 A2 variant. Nonsynonymous mutations are presented in bold face within each codon

The mutations were previously reported by [1]Bonsing et al. (1988), [2]Farrell et al. (2004) and [3]not yet defined

to the mature protein reported by Bonsing et al. (1988) and Farrell et al. (2004). In this study, sequence analysis detected five known casein beta variants, namely *A1, A2, B, F* and *I* and one new variant that has not been defined yet (Figure S1 in ESM). The *A2* allele differs from *A1* resulting in only one amino acid change at position 67 that led to the change from Pro (CCT) to His (CAT). However, in addition to position 67, the *B* and *F* alleles are responsible for substitution of amino acids at positions 122 and 152 corresponding to *A2* allele of *CSN2* gene. Mutation at codon 122, which involves C→G transition, results in the substitution of Ser (AGC) (*A2* allele) by Arg (AGG) (*B* allele). Similarly, *F* allele involves CCT→CTT substitution at codon position 152 that results in the substitution of Pro (*A2*) by Leu (*F*). Moreover, the difference between *A2* and *I* alleles is only due to one nonsynonymous mutation at codon position 93 that substitutes Met (ATG) by Leu (CTG). Notably, the *B* allele was found as a common variant in the studied population whereas the proportions of *F* and *I* alleles

https://doi.org/10.17221/206/2021-CJAS

were quite low. Besides, one variant was identified in zebu at codon position 197, which results in the substitution of Val (GTA) to Ala (GCA) and was reported for the first time in our study.

Similar to the present study, Massella et al. (2017) detected five variants (*A1, A2, B, F, I*) in Holstein Friesian and Braunvieh cattle populations of Italy. Sodhi et al. (2018) found only *A2* and *B* alleles in Indian Ladakhi cattle and is consistent to this study. Moreover, Saran et al. (2019) found *A2* allele as a primitive type in four Indian zebu breeds, namely Rathi, Sahiwal, Kankrej and Tharparkar, which supports our findings. In another investigation, Dai et al. (2016) also reported the presence of five variants (*A1, A2, A3, B* and *I*) in the Chinese Holstein population that conforms with present findings except the *A3* allelic variant which was absent in the investigated samples of this study. In contrast to our findings, Chessa et al. (2013) identified *I* as a common variant in Italian Holstein Friesian and Red Pied and in Dutch Holstein Friesian. Importantly, substitution of Val to Ala at position 197 is due to a new allele in PC zebu cattle of Bangladesh which has not yet been reported in any other cattle population. This mutation might be breed or population specific. Altogether, the types and distribution of allelic variants differ largely between indicine and taurine populations worldwide.

Genotyping of *A1* and *A2* allelic variants

Based on amino acid position 67, allele specific PCR (AS-PCR) was adopted in this study to differentiate between *A1* and *A2* alleles directly from the gel image (Figure 1). In AS-PCR genotyping, all individuals possess a common fragment of 277 bp in length while *A1A1*, *A2A2* and *A1A2* genotypes are represented by allele specific fragments of 209 bp, 108 bp and 209 + 108 bp, respectively (Figure 1). The genotype and allele frequencies of the casein beta gene in zebu and crossbred cattle populations are presented in Table 5. The mean *A1A1*, *A1A2* and *A2A2* genotype frequencies were found 0.02, 0.16 and 0.82, respectively, in six zebu populations. The mean *A1* and *A2* allele frequencies were 0.10 and 0.90, respectively. The undesirable *A1A1* genotype was found only in two individuals from RCC and DES populations. The favourable *A2A2* genotype frequencies differed from 0.68 to 0.92 among the zebu cattle populations. However, 20 individuals out of 125 were heterozygous (*A1A2*) with frequencies ranging from 0.08 to 0.26. On the other hand, in case of Holstein-zebu crossbreds, the mean *A1A1*, *A1A2* and *A2A2* genotype frequencies were 0.14, 0.50 and 0.36, respectively. The allelic frequencies of *A1* and *A2* were 0.39 and 0.61.

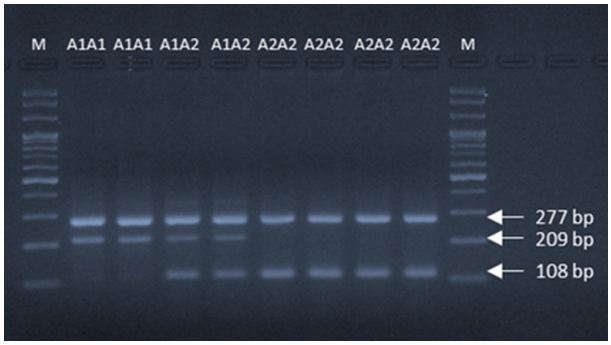

Figure 1. Allele specific genotyping of *A1* and *A2* allelic variants of casein beta gene

Line arrows = expected fragment sizes; M = 100 bp size marker

The genotypic and allelic frequencies of *A1* and *A2* variants obtained in zebu cattle populations of Bangladesh are in agreement with the previous reports of Sodhi et al. (2018) and Kumar et al. (2019). Kumar et al. (2019) found only two types of genotypes in Sahiwal cattle of India with frequencies of 0.13 (*A1A2*) and 0.87 (*A2A2*), respectively. Similarly, Sodhi et al. (2018) reported the frequencies of *A1A2* and *A2A2* genotypes to be 0.21 and 0.79, respectively, in Ladakhi cattle. However, Patel et al. (2019) identified three different genotypes as *A1A1* (0.01), *A1A2* (0.34) and *A2A2* (0.65) in Gir cattle of India while the corresponding *A1* allele frequency (0.18) was found much higher than in the earlier reports. In addition, previous reports mentioned the existence of low frequent *A1* allele in different zebu populations that ranged between 0.00 and 0.11 in Ongole, Malanad Gidda, Kherigarh, Kasargod, Achai and Lohani (Mishra et al. 2009; Ganguly et al. 2013; Ramesha et al. 2016; Ayaz et al. 2022) that support the present findings. Besides, Mishra et al. (2009) reported the absence of *A1* allele in the zebu breeds of Gir, Sahiwal, Red Sindhi, Kangayam, Nimari, Red Kandhari, Malvi, Amrit Mahal, Kankrej, Hariana, Mewati, Tharparker and Rathi and it is inconsistent with this study. Altogether, our findings suggested the almost complete fixation of *A2* allele in the zebu cattle populations of Bangladesh and therefore, the *A2A2* genotypic frequency was pre-

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Table 5. Genotype and allele frequencies of *CSN2* in zebu and crossbred cattle populations of Bangladesh

Breed/type	Genotype frequency*			Allele frequency	
	A1A1	*A1A2*	*A2A2*	*A1*	*A2*
Pabna	0.00 (0)	0.08 (2)	0.92 (22)	0.04	0.96
Red Chittagong	0.03 (1)	0.13 (4)	0.83 (25)	0.10	0.90
Sahiwal	0.00 (0)	0.17 (2)	0.83 (10)	0.08	0.92
Munshiganj	0.00 (0)	0.15 (3)	0.85 (17)	0.08	0.92
Non-descript Deshi	0.05 (1)	0.26 (5)	0.68 (13)	0.18	0.82
North Bengal Grey	0.00 (0)	0.20 (4)	0.80 (16)	0.10	0.90
Total (zebu)	0.02 (2)	0.16 (20)	0.82 (103)	0.10	0.90
Holstein-zebu crossbred	0.14 (19)	0.50 (66)	0.36 (48)	0.39	0.61

*Values in the parentheses indicate the number of samples investigated

dominantly higher than those of *A1A2* and *A1A1* genotypes.

It is notable to mention that limited information is available on *Bos taurus* × *Bos indicus* crossbred cattle for comparison with present results. Similar to our findings, Patel et al. (2019) reported that the frequencies of *A1A1, A1A2* and *A2A2* genotypes were 0.09, 0.56 and 0.34, respectively in the HF × Kankrej crossbred population and the corresponding *A1* and *A2* allelic frequency were 0.38 and 0.62, respectively. Likewise, Malarmathi et al. (2014) reported the proportion of *A1A1, A1A2* and *A2A2* genotypes to be 0.17, 0.46 and 0.37, respectively, in HF crossbred cattle of Tamil Nadu, India. The frequencies of the three genotypes were 0.56 (*A1A1*), 0.26 (*A1A2*) and 0.18 (*A2A2*) in Sahiwal × Friesian crossbreds of Pakistan (Ayaz et al. 2022). The *A1* allele frequency 0.46 was found in Vechur crossbred cattle (Muhammed and Stephen 2012) and is consistent with this finding. Miluchova et al. (2014) reported all three genotypes as 0.14 (*A1A1*), 0.46 (*A1A2*) and 0.40 (*A2A2*) in Holstein cattle, which supports our findings. Altogether, gene and genotype frequencies are breed or population specific and several factors like number of samples investigated, mutation, mating system and genetic drift largely affect the genetic constitution of a population (Falconer and Mackay 1996).

Different methods like PCR-RFLP, AS-PCR and sequencing have been employed for genotyping of the most frequent *A1* and *A2* allelic variants of *CSN2* gene (Dai et al. 2016; Massella et al. 2017; Kumar et al. 2019; Patel et al. 2019). Among them, AS-PCR is one of the rapid, cost effective and precise methods for the screening of *A1* and *A2* genotypes in different cattle populations worldwide. In this study, the newly designed AS-PCR method could discriminate allelic variants precisely and this simplified the genotyping protocol adopted in this study. This would be a promising tool to monitor the flow of undesirable alleles in the breeding herds. Furthermore, it could also be used in the commercial crossbreeding program particularly to screen the undesirable *A1* allele from the Holstein derived crossbred populations in order to get better quality milk in Bangladesh.

CONCLUSION

The genetic variabilities in the coding sequences of *CSN2* gene were analysed among the zebu and crossbred cattle populations of Bangladesh. The detected non-synonymous mutations defined five different allelic variants (*A1, A2, B, F* and *I*) in the studied samples. Sequence analysis revealed the predominance of *A1* and *A2* alleles over other alleles in taurine and zebu cattle, respectively. *A2A2* genotype was predominantly higher in zebu populations with *A2* allele frequency of 0.90. In contrast, half (50%) of the Holstein derived crossbred population was found heterozygous (*A1A2*) while only 36% of them were the homozygous *A2A2* genotype. The newly designed AS-PCR could be used as a method for the screening of breeding animals for their *A1* and *A2* allelic variants, and thereby it could possibly reduce the proportion of undesirable *A1* allele, particularly from the crossbred herds. This will eventually lead to production of better quality milk from the crossbred dairy herds.

https://doi.org/10.17221/206/2021-CJAS

Acknowledgement

The authors acknowledge with gratitude for the cordial help of dairy cattle farmers for providing the necessary information during blood sampling.

Conflict of interest

The authors declare no conflict of interest.

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https://doi.org/10.17221/206/2021-CJAS

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Received: December 8, 2021
Accepted: August 17, 2022
Published online: August 31, 2022