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Sequence based typing for BoLA-*DQA1* alleles in Indian zebu and its crossbred populations

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ABSTRACT

Polymorphism in the major histocompatibility complex of the bovine, which is referred to as bovine leukocyte antigens (BoLAs), was intensively investigated for identifying marker(s) for bovine diseases and immunological traits. This approach requires identifying and documenting the allelic diversity of BoLA among different breeds of cattle using PCR-SBT technique. In this study, we could standardise the most useful approach of unravelling polymorphism in *DQA1* exon 2 of cattle using PCR-sequence based typing (SBT). We could identify 16 different *DQA1* alleles in 43 zebu and crossbred cattle, out of which 26 were homozygous and 17 were heterozygous. PCR-SBT has proved to be the most useful method for exploring the allelic polymorphism in *DQA1* gene in zebu and its crossbred cattle in our study. Further use of this technique is equally appropriate for all kinds of traits i.e. production, reproduction and growth type of traits and beneficial for establishing new breeding objectives.

Key words: BoLA II, *DQA1*, Major histocompatibility complex, Sequence based typing

The major histocompatibility complex (MHC) is important for induction and regulation of immune response and many of its genes have been intensively evaluated as candidate marker for various bovine diseases and immunological traits due to their allelic diversity (Takeshima *et al.* 2006, Behl *et al.* 2007). In cattle, the MHC is called bovine leukocyte antigen (BoLA). A major rearrangement in antigen binding groove within the class II region has led to the division of BoLA region into 2 distinct sub-regions of chromosome 23 and is characteristic of other species of ruminants like sheep, goat and buffalo (Amills *et al.* 1998, Andersson and Rask, 1988, Band *et al.* 1998). The class IIa sub-region contains the functionally expressed *DR* and *DQ* genes, while the class IIb contains genes of undefined status including *DYA, DYB, DMA, DMB, DOB, TAP1, TAP2, LAP2,* and *LMP7*. The *DQ* gene has been extensively used for class II haplotypes analysis and elucidating the detail of disease association or economic trait in most of the farm animals (Andersson and Rask 1988, Sigurdardottir *et al.* 1992, Marello *et al.* 1995, Ballingall *et al.* 1997, Russell *et al.* 1997, Snibson *et al.* 1998).

The Class II region of the MHC is highly polymorphic and it encodes for both α (DQA) and â (DQB) chains that form the Class II heterodimer. In cattle, most haplotypes carry duplicated *DQ* genes (Ballingall *et al.* 1997, Russell *et al.* 1997). Cattle have approximately 51 characterized alleles of *DQA* gene; out of these, 25 alleles belong to *DQA1* subgroup (Miyasaka *et al.* 2011). Most of the diversity in this gene has been studied using PCR-RFLP, whereas recent advances in sequencing technology has yielded a new approach called sequence based typing (SBT) where PCR products are directly sequenced to reveal greater amount of polymorphism. In India, there has not been any report on genetic polymorphism of *DQA1* gene exon 2 in cattle. In the present study, considering the documented allelic diversity and immunological importance of *DQA1* as it expresses more restriction element for generating protective immunity, exon 2 region is selected for study. In the present study, we report PCR-SBT system to analyse genetic diversity in Indian Zebu and its crossbred cattle.

MATERIALS AND METHODS

Experimental Animals: Hariana cattle (10) were from organized herd at Mathura located in the tropical region at 27°272 N, 77°432 E and having an elevation of 174 m (570 ft) above the mean sea level (msl). Tharparkar (10) and crossbred (10) cattle were from IVRI Izatnagar (Bareilly) herd that is located in the tropical region at 28°102 N, 78°232 E, and 81 m (266 ft) above mean sea level. Crossbred were developed by crossing indigenous Hariana cattle with Holstein Friesian and either Jersey or Brown Swiss, hereafter called crossbred (herd 1). Crossbred (13) hereafter called herd 2 maintained at Mukteshwar (Nainital, Uttarakhand) was located in the temperate Himalayan region of India at 29°282 203 N, 79°382 523 E, and 2,171 metres (7,123 feet) above msl. Crossbred (herd 2) was developed by crossing Hariana cattle with Holstein

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Friesian alone or Holstein Friesian in combination with Jersey. Exotic inheritance level was more than 50%.

DNA isolation and PCR amplification of BoLA DQA1 gene: Blood samples were collected from all 43 animals and genomic DNA was isolated by DNA extraction kit. Amplification of BoLA *DQA1* gene was performed by nested PCR reaction with two sets of primers specific for *DQA1* exon 2 as reported by Takeshima *et al.* (2007). Optimization of the annealing temperature and PCR protocol was done for the amplification of 355 bp product of partial gene. A set of primer (DQAintL2: CACAAATG AAGCCCACAATG and DQA1-677R: CCCTAGGGAAA AGGGAGTGA) was used for amplification of 426 bp fragment containing exon 2 and flanking intron sequences. For the first round, 15 cycles were used. The PCR reaction mixture contained optimized concentration of Taq buffer 10x, 1.5 mM of $MgCl_2$, 10 mM of dNTPs, 20 picomole of primers, 20 ng of genomic DNA, and Taq DNA polymerase. Following initial denaturation at temperature 94ºC for 2 min, amplification cycle consisted of denaturation at 94ºC for 20 sec, annealing at 54ºC for 20 sec and extension at 72ºC for 40 sec. After 15 cycles, a final extension at 72ºC for 4 min was done.

The product of first PCR reaction was used as template for second round of PCR amplifying the DQA1 using second set of primers (DQAintL3: GCCCACAATGTTT GATAGTC and DQA1ex2REV ver2.1: GGGRACACATA CTGTTGGTAGA). The 249 bp of exon 2 and 94 bp of the 5'end of intron 1 and 12 bp of 3'end of intron 2 were amplified using fully nested PCR. The product of first PCR reaction (25 µl) served as template for the second, where the final volume was 50 µl. Reaction mixture contained optimized concentration of Taq buffer 10x, 1.5 mM of $MgCl_2$, 10 mM of dNTPs, 20 picomole of primers, 20 ng of genomic DNA, and Taq DNA polymerase. Following initial denaturation at temperature 94ºC for 2 min, amplification cycle consisted of denaturation at 94ºC for 20 sec, annealing at 54ºC for 20 sec and extension at 72ºC for 40 sec. After 35 cycles, a final extension at 72ºC for 4 min was done.

Sequence based typing: PCR products were purified using PCR purification kit, samples were submitted for sequencing to a commercial unit. The sequences were obtained using forward and reverse primers ACAATGTTTGATAGTCAATTTC and GGGRACACAT ACTGTTGGTAG, respectively (Takeshima *et al.* 2007). Chromatogram base calling was performed using Chromas Lite software. Allele assignment was done with the help of National center for biotechnology information (NCBI) - Basic local alignment search tool (BlastN). Along with BlastN, we also confirmed the alleles by alignment of nucleotide sequences of BoLA *DQA1* with W1 clones (Van der Poel *et al.* 1990) for intron 1 and exon 2, respectively. Further confirmation of the allelic patterns was done using predicted amino acid sequences of the α 1 domain of exon 2 of already known alleles. Three different approaches were used for validating the obtained sequences. 100% matching of the obtained sequences was mandatory before assigning allele name to the sequences.

RESULTS AND DISCUSSION

A total of 16 *DQA1* alleles were found among the 4 cattle population using PCR-SBT and BLAST analysis in the 43 animals of 4 cattle subpopulations studied. Out of these, 26 were homozygous and 17 were heterozygous for these 16 alleles. This is the first report of PCR-SBT study for *DQA1* gene in cattle in India. Alignment of nucleotide sequences of BoLA *DQA1* intron 1 of these alleles with the earlier reported W1 genomic clone was done for further

Table 1. Distribution o f BoLA-DQA1 alleles detected in 4 subpopulations of cattle

BoLA DQA1 Alleles	Number of distinct alleles	#Breed	Breed in which alleles were already reported
*0101	6	H, CH1	Holstein Friesian[b], N'Dama[c,d], BF[a], DB[a]
*0102	5	H, CH1, CH2	BF[a] , N'Dama[c]
*0103	1	CH1	DR[a], JE[a], N 'Dama[c]
*0203(1)	1	H	BF[a], DB[a], JE[a], BF× HE[a]
*0203(2)	1	H	BF[a], DB[a]
*0204	9	H, CH1	BF[a], JE[a], BF×AY[a] , N'Dama[c], Boran[c]
*0301	1	CH1	DR[a], JE[a] , Boran[c]
*0801	4	T	JE[a], N'Dama[c,d]
*10011	20	H, CH1, CH2	DB[a], BF×AY[a] , N'Dama[b], Boran[c,d]
*10012	3	H, CH2	BF[a], DB[a], DR[a]
*12011	3	H	BF[a], DB[a], Holstein Friesian[c]
*12012	7	T, H, CH1, CH2	BF[a], DB[a], DR[a]
*12021	6	T, H, CH2	BF[a], DB[a], DR[a], JE[a] , Japanese black[e]
*1203	2	CH1	DR[a], N'Dama[d]
*1401	6	T, H, CH2	DR[a], N'Dama[c], Boran[c,d]
*1402	11	T, H, CH2	

#T, Tharparkar; H, Hariana; CH1, crossbred herd1; CH2, crossbred herd 2; [a] published by Takeshima et al. (2007): AY, Ayrshire ; BF, British Friesian; DB, Danish Black Pied; DR, Danish Red; HE, Hereford; HF, Holstein Friesian; JE, Jersey; LM, Limousin; [b]Published by Russell *et al.* (1997). [c]Published by Gelhaus *et al.* (1999). [d]Published by Ballingall *et al.* (1990). [e]Published by Nishino *et al.* (1995).

confirmation. Enormous polymorphism for *DQA1* gene in present study is in congruence with the earlier reports (Table 1) that used PCR-SBT to explore the allelic diversity of *DQA1* gene in cattle. Takeshima *et al*. (2007) who established PCR-SBT for *BoLA-DQA1* exon 2 revealed 35 heterozygous and 16 homozygous cases for 15 distinct BoLA *DQA1* alleles among various exotic cattle population. Schwab *et al.* (2009) reported 10 different *DQA1* alleles in Holstein cattle by PCR-SBT. In the Japanese black and Holstein cattle, Miyasaka *et al.* (2011) reported 15 types of *DQA1* alleles using PCR-SBT. Similarly, Miyasaka *et al.* (2012) reported 22 BoLA *DQA1* alleles by PCR-SBT.

Confirmation of BoLA DQA1 alleles: In the present study, allele identification was done using BLAST online software. The alleles were further confirmed by crosschecking with earlier reports. We have used three approaches for confirmation of the observed alleles in the study. We have analysed the sequences and aligned them with W1 genomic clone (Van der poel *et al.* 1990) for intronic region based confirmation and confirmation by alignment with previously reported alleles (Takeshima *et al.* 2007). It was then followed by confirmation at nucleotide level of exon 2 of W1 genomic clone (Van der poel *et al.* 1990) and then by predicted amino acid level similarity.

All the 16 alleles obtained in present study were overall 97.3–100 % identical (Fig 1.1) at nucleotide positions – 74 to – 1 of intron1 to the sequence of W1 genomic clone (Van der poel *et al.* 1990). These results were in agreement with the previously published results of Takeshima *et al.* (2007), as all the 16 alleles in present study were 100% matching with the allelic sequences reported by Takeshima *et al.* (2007). Alignment of homozygous sequences of 9 alleles obtained in the present study at the nucleotide level and amino acid level in exon 2, revealed that all the 9 alleles were 93.8 –100 % identical (Fig 1.2) at nucleotide level of exon 2, and 86.3 – 100% identical (Fig 1.3) at the amino acid level coded by exon 2. Among 9 alleles, BoLA-*DQA1**1203 allele showed 100 % identity at nucleotide as well as amino acid level coded by exon 2. All homozygous nucleotide sequences (9) of present study were matching in 100% conformity with the previously published alleles of BoLA *DQA1* (Takeshima *et al.* 2007) at nucleotide

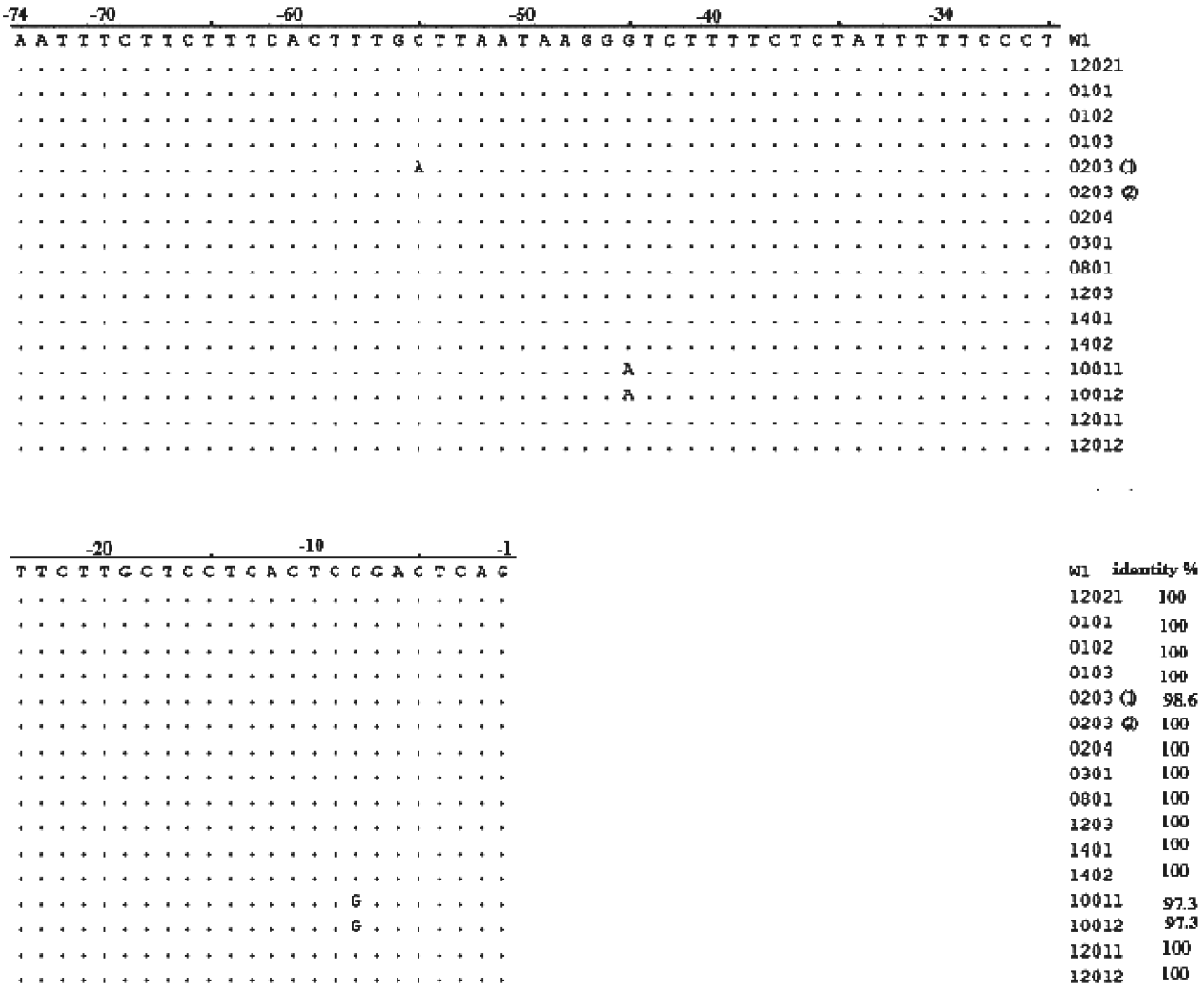

Fig. 1.1 Alignment of nucleotide sequences of identified BoLA DQA1 intron 1 with W1 genomic clone.

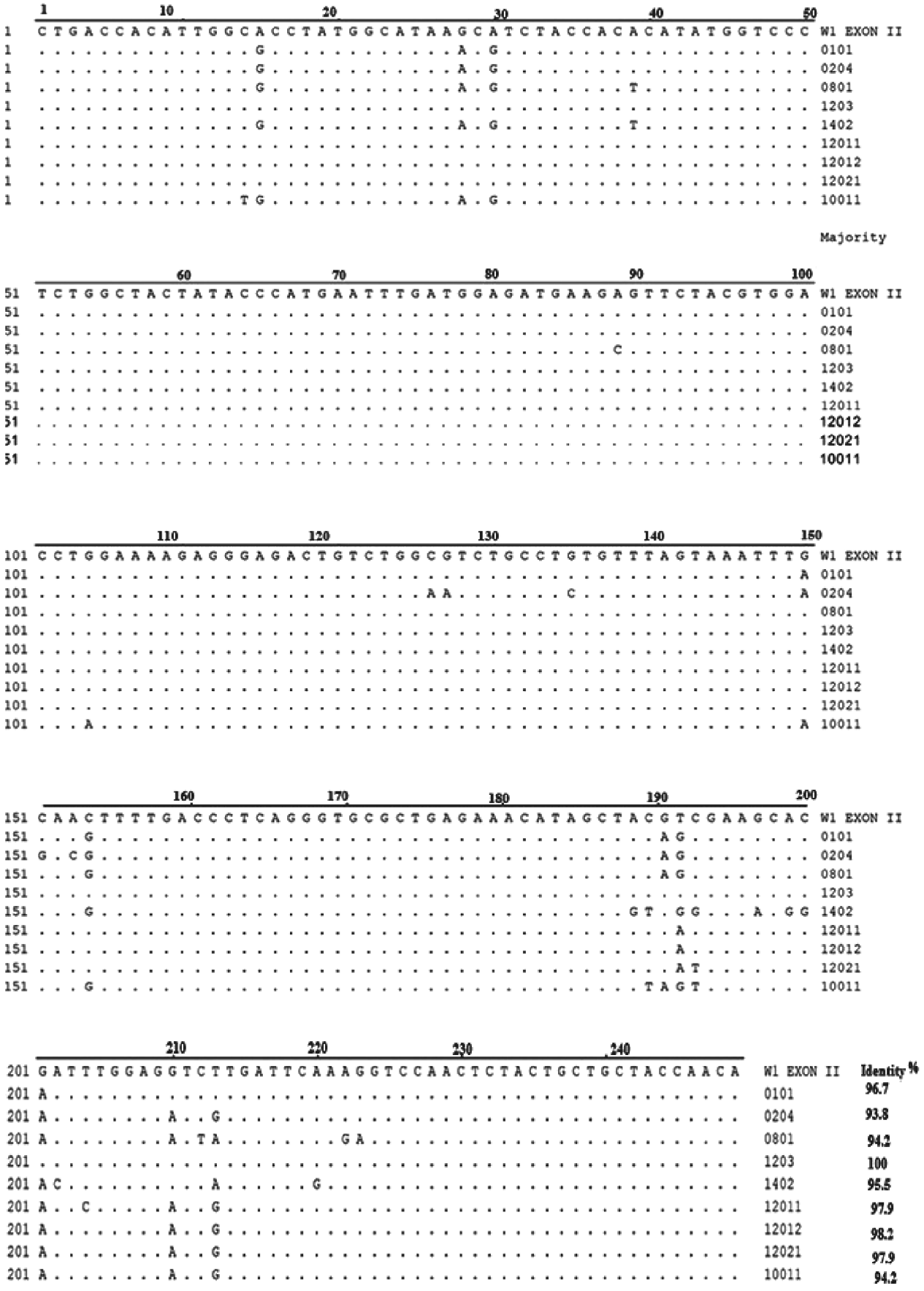

Fig. 1.2 Alignment of nucleotide sequences of identified BoLA DQA1 exon 2 with W1 genomic clone.

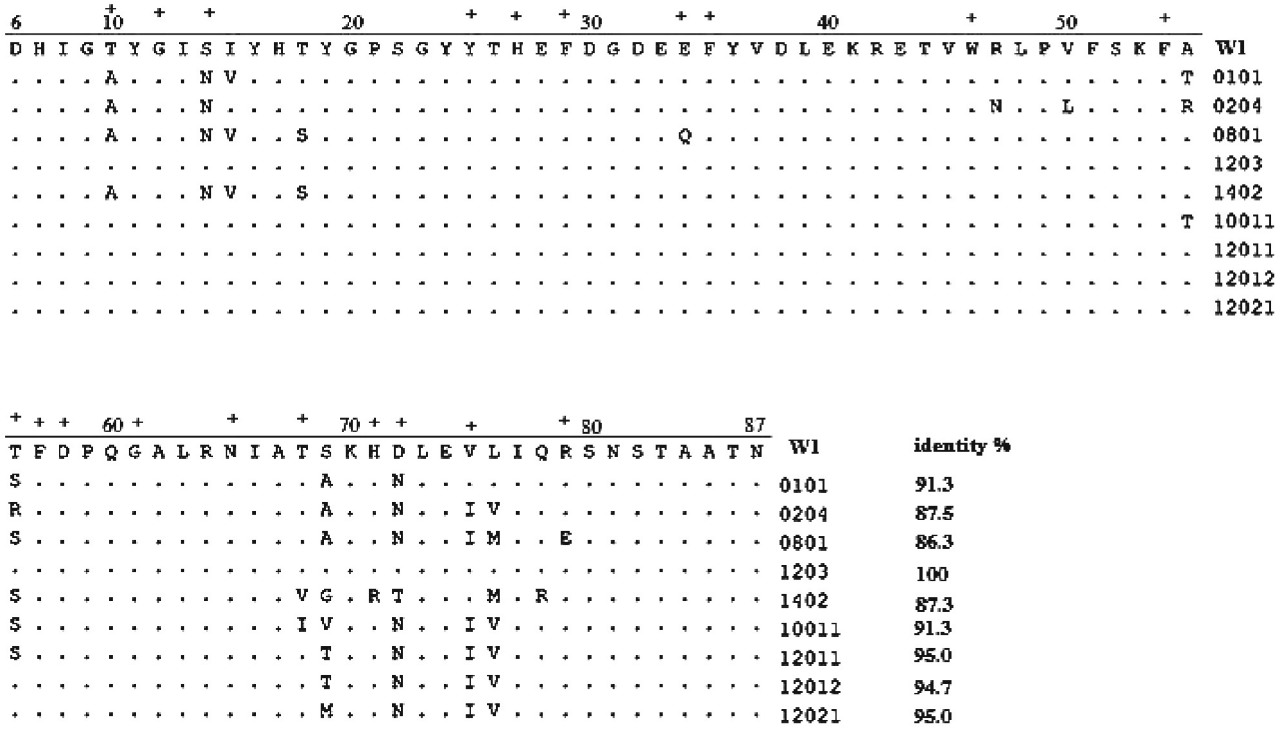

Fig. 1.3 Alignment of predicted amino acid sequences of BoLA DQA1 coded by exon 2 of alleles with W1 genomic clone.

The nucleotide position numbers (Fig 1.1 and 1.2), amino acid position numbers in the mature protein (Fig 1.3), and putative antigen recognition sites ('+' in Fig 1.3) are taken from the report of Brown *et al.*(1993) and are indicated at the top of the sequences. Dots indicate sequence identity with respect to the sequence of the W 1 BoLA-DQA1genomic clone (M30117), reported by Van der Poel *et al.* (1990). Homology scores also refer to this genomic clone in bold face.

positions – 74 to – 1 of intron1, nucleotide level in exon 2 and amino acid level coded by exon 2 of W1 genomic clone.

This technique has many advantages as it can reveal the complete polymorphism in the population along with revealing the heterozygosity. PCR-SBT for BoLA-*DQA1* as developed by Takeshima *et al.* (2007) provides full information about the gene segment studied, saves much time and work to obtain the information. This method is better as compared to cloning and sequencing as the problem of misincorporations of bases for heterozygous individuals are diluted in direct sequencing and are therefore expected to be at background level (Takeshima *et al.* 2007). Therefore this method also minimizes the risk of false homozygosity or false heterozygosity.

To conclude, SBT is the most suitable approach for identifying the allelic diversity for *DQA1* in cattle that surpasses all other past approaches such as RFLP for genotyping. In the populations studied, we observed various alleles out of which a few can assist for Marker assisted selection (MAS), if data on various disease aspects is available. Not only this but SBT has also revealed in-depth knowledge about the genetic relationship within and among the populations studied. Such information helps us to understand the evolutionary association between the breeds and their significance in the present context.

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