

BOLA-DRB3 GENE POLYMORPHISM IN JAFFARABADI AND MEHSANI BUFFALOES AS REVEALED BY PCR-RFLP

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A number of serological, biochemical and molecular methods have been used to investigate the genetic diversity of Major histocompatibility complex (MHC) genes in cattle. Class II BoLa-DRB3 gene exon2 has been found to be highly polymorphic (Siguardarottir *et al.*, 1988, 1991). So far, more than 63 DRB3 alleles have been identified by DNA sequencing (Russell *et al.*, 1997); BoLa home page <http://www2.ri.bbsrc.ac.uk/bola/>). Similar information on DRB3 polymorphism in buffaloes is lacking. Present study reports the DRB3 gene polymorphism in exon2 in Jaffarabadi and Mehsani buffaloes as revealed by PCR-RELP.

were digested with five units of Hae III and Rsa I in 30 ml reaction volume. The restriction fragments were resolved on 2-3% agarose gel and visualized by ethidium bromide staining. The fragments were also resolved on 6% urea-polyacrylamide sequencing gel and visualized by silver staining. Molecular weight markers, 100 bp and 50 bp ladders were used as size standard. Hae III and Rsa I RFLP patterns were typed as described by van Eijk *et al.*, (1992); updated by Gelhaus *et al.*, (1995) and Maillard *et al.*, (1999). Samples with ambiguous restriction patterns or with patterns not confirming to known types were excluded from the analysis.

Materials and Methods

Genomic DNA was extracted from 50 Jaffarabadi and 50 Mehsani buffaloes (John *et al.*, 1991). A 284 bp exon2 segment of DRB3 was amplified by heminesting PCR using primers HLO-30, -31 in the first round and HLO-30, -32 in the second round of PCR as described elsewhere (Acharya *et al.*, in press).

PCR-RELP analysis : PCR products

Results and Discussion

Hae III restriction patterns : On Hae III digestion of 284-bp DRB3 exon2 segment, restriction fragments of 219, 190, 167, 117, 65 and 52 bp were generated in different combination. The RFLP patterns were typed as a to e. Four patterns a, b, d and e in seven different combination (aa, ab, ae, bb, be and ee) in Jaffarabadi and four patterns a, b, d, e in nine different combinations (aa, ab, ad, bb, bd, be, dd, de and ee) were

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identified in Mehsani (Fig. 1). Owing to difficulty in appreciating minor variation in fragment sizes, patterns *c*, *f*, *g*, *h*, *i* could not be ascertained and might be typed as *a* or *e*. The pattern *a* to *e* occurring in different combinations in Jaffarabadi and Mehsani

buffaloes are summarized in Table.

There was no breed specific pattern and all the patterns prevailed evenly in different combinations.

Table - Frequencies of various BoLA-DRB3 Hae III RFLP patterns in Jaffarabadi and Mehsani buffalo

Breed	Hae III RFLP Pattern Combinations										RFLP Patterns			
	aa	ab	ad	ae	bb	bd	be	dd	de	ee	a	b	d	e
Jaffarabadi (n = 48)	0.229 (11)	0.229 (11)	-	0.167 (8)	0.271 (13)	-	0.042 (2)	-	0.021 (1)	0.042 (2)	0.427 (41)	0.408 (39)	0.010 (1)	0.156 (15)
Mehsani (n = 43)	0.070 (3)	0.233 (10)	0.023 (1)	-	0.465 (20)	0.093 (4)	0.023 (1)	0.023 (1)	0.047 (2)	0.023 (1)	0.198 (17)	0.640 (55)	0.105 (9)	0.058 (5)

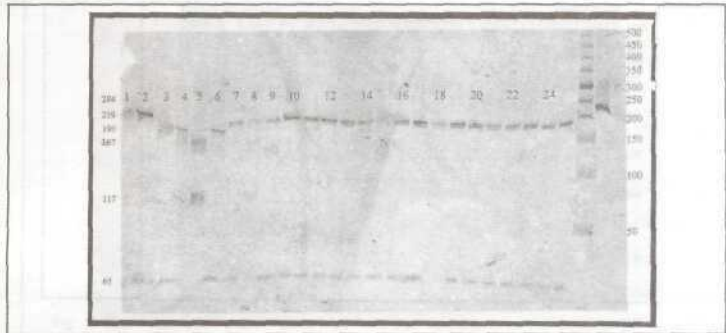


Fig. 1 Hae III restriction pattern generated from 284 pb PCR amplified BoLa DRB3 exon2 fragment electrophoresed on 6% urea-polyacrylamide gel

- Lane 1, 2, 7, 10-25 restriction 'bb' (219 bp, 65 bp)
 4, 6 restriction pattern 'dd' (190 pb, 65 pb)
 5 restriction pattern 'ee' (167 pb, 117 bp)
 8, 9 restriction pattern 'ab' (219 bp, 65 bp)
 26 50 bp DNA ladder
 27 PCR Product (284 bp)

BoLA and DRB3 gene polymorphism in buffaloes

Van Eijk *et al.*, (1992) for the first time described six *Hae III* restriction patterns, *a* to *f* in European breeds of cattle. Later on Gelhaus *et al.*, (1995) explored two new *Hae III* restriction patterns *g* and *h* in DRB3 exon2 segment from H.F. and African cattle breeds, N'Dama and Boran. Maillard *et al.*, (1999) observed all the *Hae III* patterns in Zebu Brahman (*Bos indicus*) cattle that were previously described in European breeds. Arvindakshan and Nainer (1999) investigated BoLA-DRB3 polymorphism in Jersey crossbred and Ongole animals by PCR-RFLP of 304 bp exon2 segment. They reported seven *Hae III* patterns in Jersey crossbred and four patterns in Ongole with a new pattern '*i*' in Jersey crossbreds and Ongole.

Rsa I restriction patterns : *Rsa I* digestion of DRB3 exon2 generated several restriction fragments of variable size viz., 284, 234, 180, 143, 141, 111, 104, 93, 78, 69, 63, 54, 50, bp etc. in different combinations. The patterns were typed as *a* to *y* (Fig. 2). The RFLP analysis of *Rsa I* digest was very complex as it yielded a total of 23 different pattern combinations. These are summarized in Tables 2 and 3.

Digestion with *Rsa I* resulted in 10 patterns (*d, f, g, i, k, m, n, o, s, t*) in 15 different combinations in Jaffarabadi and seven restriction patterns (*a, c, d, g, i, k, l, o*) in 12 different combinations in Mehsani. Among these the pattern combinations



Fig. 2. *Rsa I* restriction pattern generated from 284 bp PCR amplified BoLA DRB3 exon 2 fragment electrophoresed on 6% urea-polyacrylamide gel

Lane 1, 10, 13, 17, 20, 21	restriction pattern 'll' (234 bp, 50 bp)
2, 5, 22	restriction pattern 'ff' (141 bp, 54 bp, 50bp)
3, 11, 24, 28	restriction pattern 'fl' (231 bp, 141 bp, 54 bp, 50bp)
4, 9, 23	restriction pattern 'oo' (284 bp)
6	restriction pattern 'fi' (180 bp, 141 bp) ambiguous
7	restriction pattern 'kk' (156 pb, 78 bp, 50bp)
8	restriction pattern 'fi' (234 pb, 141bp) ambiguous
12	restriction pattern 'os' (284 bp, 141 bp, 93 bp, 50bp)
16, 18	restriction pattern 'fo' (284 bp, 141 bp, 54 bp, 50bp)
25	100 bp DNA ladder

Underlined fragments not seen as the gel pieces lost during gel drying.
Lanes 14, 15 and 19 samples not loaded

Table 2 - Frequencies of various BoLA-DRB3 *Rsa* / RFLP pattern combinations in Jaffarabadi and Mehsani buffalo

Sr. No.	Combination	Jaffarabadi	Mehsani	Sr. No.	Combination	Jaffarabadi	Mehsani
		Frequency (n)	Frequency (n)			Frequency (n)	Frequency (n)
1	<i>bb</i>	-	0.063 (1)	13	<i>'s</i>	0.052 (1)	0.062 (1)
2	<i>by</i>	-	0.125 (2)	14	<i>lw</i>	0.157 (3)	-
3	<i>dd</i>	0.053 (1)	-	15	<i>mm</i>	0.052 (1)	-
4	<i>fl</i>	0.053 (1)	-	16	<i>oo</i>	0.052 (1)	-
5	<i>gg</i>	-	0.188 (3)	17	<i>oy</i>	-	0.062 (1)
6	<i>gh</i>	0.053 (1)	-	18	<i>ss</i>	0.116 (2)	0.125 (2)
7	<i>gj</i>	0.053 (1)	-	19	<i>sw</i>	0.052 (1)	-
8	<i>gl</i>	0.053 (1)	0.063 (1)	20	<i>yy</i>	-	0.062 (1)
9	<i>gm</i>	0.053 (1)	-	21	<i>ww</i>	0.105 (2)	-
10	<i>go</i>	-	0.063 (1)	22	<i>nw</i>	0.052 (1)	-
11	<i>gy</i>	-	0.063 (1)	23	<i>ny</i>	-	0.062 (1)
12	<i>ll</i>	0.052 (1)	0.062 (1)				

Table 3 - Frequencies of various BoLa-DRB3 *Rsa* / RFLP patterns in Jaffarabadi and Mehsani buffalo

Breed	<i>Rsa</i> I RFLP Patterns												
	b	d	f	g	h	j	i	m	n	o	s	w	y
Jaffarabadi (n=19)		0.053 (2)	0.026 (1)	0.105 (4)	0.026 (1)	0.026 (1)	0.211 (8)	0.105 (4)		0.053 (2)	0.158 (6)	0.237 (9)	
Mehsani (n=16)	0.125 (4)			0.281 (9)			0.125 (4)		0.031 (1)	0.063 (2)	0.156 (5)		0.219 (7)

common to both the buffalo were *gl* (234, 141, 104, 50 and 39bp), *ll* (234 and 50bp), and *ls* (234, 141, 93 and 50 pb). RFLP patterns *g*, *i*, *o* and *s* were common to both the buffalo breeds.

Because of complexity of the restriction patterns and ambiguity in

correctly identifying the size of the *Rsa* I restriction fragments, 19 samples in Jaffarabadi and 16 samples in Mehsani could only be typed. Remaining samples could not be assigned to any known pattern and were excluded from the analysis. Many of these might be revealing a new variant pattern, but in absence of supporting

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evidence, sequencing this was not attempted.

Van Eijk *et al.*, (*loc. cit.*) for the first time described 19 *Rsa I* restriction patterns (a to s) in European cattle. Gelhaus *et al.*, (*loc. cit.*) described two new *Rsa I* restriction patterns, t and u in H.F. and African breeds, N'Dama and Boran. Maillard *et al.*, (*loc. cit.*) recently identified four new patterns v, w, x and y in *Bos indicus* cattle. Arvindakshan and Nainer (*loc. cit.*) investigated BoLA-DRB3 polymorphism in Jersey crossbred and Ongole cattle by PCR-RFLP. They observed only 11 restriction patterns, 8 patterns in 14 different combinations in Jersey and 7 patterns in 10 different combinations in Ongole. From the available literature it seems, on DRB3 exon2 RFLP patterns are described for buffaloes.

Present study revealed several RFLP pattern combinations in buffalo sample indicative of large sequence variation and thus, high degree of polymorphism in this segment.

Summary

BoLA-DRB3 gene polymorphism was investigated in two buffalo breeds, Jaffarabadi and Mehsani by PCR-RFLP. A 284 bp BoLA-DRB3 exon2 segment was amplified by hemi-nesting PCR and was digested with *Hae III* and *Rsa I*. Digestion with *Hae III* resulted in four RFLP patterns a, b, d and e in seven different combinations in Jaffarabadi and four RFLP patterns a, b, d and e in nine different combinations in Mehsani. Pattern a and b were found to be

more frequent in both these breeds, *Rsa I* restriction patterns were very complex. The fragment interpretation revealed 10 different restriction patterns with 15 combination in Jaffarabadi and 7 different restriction patterns in 12 combinations in Mehsani. This suggests very high level of polymorphism in BoLA-DRB3 gene segment.

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