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RESEARCH ARTICLE

GENETIC DISTANCE BETWEEN TWO BREEDS OF CATTLE "HOLSTEIN- FRIESIAN AND JERSEY CROSSBRED" BY USING RAPD-PCR

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ABSTRACT

The investigation was taken up to study Genetic Distance between two breeds of cattle "Holstein and Jersey crossbred" by using Random amplified polymorphic DNA technique. This technique was carried out with 10 random primers. RAPD-PCR pattern revealed the total polymorphism loci of two breeds (67 loci), the total loci amplified in two breeds (118). The estimate of genetic distance was highest (2.21) with the primer (OP13) and the lowest (0.33) with the primer (OP15). The GD pooled over the primers was (1.40 ± 0.17) between these two breeds. The highest average percentage difference estimate (90.69) with the primer (OP15) and the lowest (19.81) with the primer (OP13), and the MAPD between two breeds estimate (54.41 ± 3.82).

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INTRODUCTION

Livestock play a vital role in agriculture. India has rich livestock diversity in terms of species, breeds and varieties adapted to different agro climatic zones and geographical regions. Approximately 70% of India farms are dependent for their livelihood on agriculture (ICAR, 2004). The cattle are one of the prime animal genetic resources of India. Among them Holstein- Friesian and Jersey are great importance in India. Molecular biology virtually gains access to the entire genome of cattle. DNA fingerprinting is an encrypted set of numbers that reflect a person's DNA makeup, which can also be used as the person's identifier. It has a wide range of applications in different aspects of medical, animal and veterinary sciences (Bhattacharya *et al*., 2008). RAPD technique was developed by Williams *et al*., 1990 (Li *et al*., 2006 and Zhang *et al*., 2002b). RAPD is an amplification of genomic DNA using at least one short oligonucleotide primer in low stringency condition resulting in multiple amplification products from loci distributed through the genome. The studies on population genetic structure and genetic variation using RAPD were claimed to be quite successful (Apostol *et al*., 1996 & Yu *et al*., 2004).

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Therefore, it is a powerful tool in DNA fingerprint analysis of various animal species, gene mapping studies, population analysis and identification of breeds.

MATERIALS AND METHODS

In the present study, a total of 20 animals of both sexes were selected randomly from two breeds of cattle viz. Holestein and Jersey, out of 20 aniamals 10 animals belonging to Holestein and 10 animals belonging to Jersey breed. The blood samples were collected aseptically into the EDTA blood collecting tubes from jugular vein. Genomic DNA was isolated from blood cells by lysis buffer. DNA samples were checked for quality by running them in 0.8% Agarose gel. Only intact DNA samples devoid of smearing were used for further analysis. The DNA concentration was calculated by measuring OD at 260 nm (1 OD_{260} =50 µg of double stranded DNA/ml).

RAPD-PCR technique

The sequence of Guanine and Cytosine (GC) contents are mentioned in Table1. The PCR reaction mixture consist of 2 µl from (DNA template, 10 mM $MgCl_2$, 10 mMdNTP), 40 ng primer, 4µl (10XPCR Buffer), 1µl (Taq DNA polymerase) and 10 µl (Distilled water). The amplification

was carried out for 30 cycles with initial denaturation at 95^0C for 5 min, second denaturation for 1 min at 94^0C, annealing at 36^0C for 1 min, extension for 2 min at 72^0C and final extension at 72^0C for 5 min. All the amplified products were separated by 72^0C and final extension at 72^0C for 5 min. All the amplified products were separated by electrophoresis in 0.8% agarose gel containing ethidium bromide and photographed under UV light.

Table 1. RAPD Primer sequences with length and Guanine-Cytosine contents

Primers	Sequence (5'---------3')	Length	GC %	Screened/
OP-01	TGGGTCCCTA	10-mer	60	Employed
OP-02	CGTCCCGGAG	10-mer	80	Screened
OP-03	GCCTACAACG	10-mer	60	Employed
OP-04	ACAGGCTGCC	10-mer	70	Employed
OP-05	TAGGCAGTCT	10-mer	50	Screened
OP-06	ACCGTCACTC	10-mer	60	Screened
OP-07	ACAGGCTCCA	10-mer	60	Employed
OP-08	GCGGTACCTT	10-mer	60	Employed
OP-09	AGTGGCTCGC	10-mer	70	Screened
OP-10	TCGAGGTCGC	10-mer	70	Screened
OP-11	AGATCCCGCC	10-mer	70	Employed
OP-12	GCCGTCCGAG	10-mer	80	Employed
OP-13	CTGAGACGGA	10-mer	60	Employed
OP-14	GCATCCACGC	10-mer	70	Screened
OP-15	CAGGATCCAG	10-mer	60	Employed
OP-16	TGGCACCTTC	10-mer	60	Employed

Band sharing frequency (BSF): Band sharing frequency were calculated in pair wise comparisons as described by Gwakisa *et al*., (1994):

BSF (between two breeds) = 2BMS / [BM + BS]

Where BMS is the number of bands common to Holstein and Jersey for a primer. BM is the total number of bands for Holstein for particular primer. Similarly BS is the total number of bands for Jersey for a primer. Average of band shring frequency was calculated by dividing the sum of BFS value of pair wise comparison by total number of pairs compared. Within breed band sharing frequency was calculated as average of band sharing frequencies within the breeds pair wise using formula:

BSF (within Holstein breeds) = 2B MI.MII / [BMi + BMii] and

BSF (witin Jersey breeds) = 2B Si.Sii / [BSi + BSii]

Where BMi Mii is the band sharing frequency within a pair of Holstein cattle and BSi Sii is the band sharing frequency wthin a pair of Jersey cattle. BMi, BMii, BSi and BSii are the total bands observed in the individual animal of Holstein and Jersey breeds respectivily.

Genetics distance (D): The genetics distance were obtained by the formula of Lynch (1991): DMS= -ln BSFMS /√(BSFM X BSFs)

Where BFSMS is the band sharing frequency between Holstein and Jersey breeds of buffalo. BSFS is the band sharing frequency within Holstein breeds. BSFS is the band sharing frequency within Jersey breeds.

Mean average percentage diffrence (MAPD) MAPD was calculated by using the following formula (Gilbert *et al*., 1990; Yukhi and O'Brien, 1990).

PD = [NMS /NM + NS] x 100

MAPD = 1/R ∑ APDi

Where NMS are the number of fragment that differed between two animals, for single primer. NM and NS are the number of band observed in individual breeds. R is the number of random primers used.

RESULT AND DISCUSSION

RAPD-PCR

The total number of bands amplified ranged from 1 to 16 Table (2 and 3). In Holstein-Friesian breed, the traits of the amplification profiles using 10 random primers have been presented Table 2. The bands varied in their size from 241-2030bp. All the 10 primers detected polymorphism among the population of cattle breeds.

Table 2. Amplification profile for different primers in Holstein-Friesian Breed

Primer	Total loci	Polymorphic	Size range (bp)	Size difference	Percent
Code	amplified	loci		(bp)	Polymorphism
OP-01	06	06	2030-425	1605	100.00
OP-03	06	05	1165-700	465	083.33
OP-04	08	07	1600-300	1300	087.50
OP-07	06	04	1500-600	900	066.66
OP-08	05	03	1300-325	925	060.00
OP-11	06	02	1450-600	850	033.33
OP-12	01	01	872-241	631	100.00
OP-13	07	02	1550-1250	300	028.57
OP-15	04	00	1400-700	700	000.00
OP-16	06	01	1500-325	1175	016.00
Total	55	33			57.60%

From the 10 random primers, a total of 55 bands were amplified and 31 of these (about57.6%) were found to be polymorphic among the population. Number of polymorphic

loci ranged from 0 to 7. OP-1 and OP-12 produced 100% polymorphic loci, while OP-15, the polymorphic loci were 0%. In Jersey breed, the traits of the amplification profiles using 10 random primers have been presented Table 2. The bands varied in their size from 325-2750bp.From the 10 random primers, a total of 63 bands were amplified out of which only 36 were polymorphic giving (about 55.33%) polymorphism. OP-04 produced 100% polymorphic loci. Number of polymorphic loci ranged from 1 to 7.

Table 3. Amplification profile for different primers in Jersey Breed

Primer Code	Total loci amplified	Polymorphicloci	Size range (bp)	Size difference (bp)	Percent Polymerphism
OP-01	08	07	2750-1900	850	087.50
OP-03	06	04	1500-1078	422	066.66
OP-04	06	06	1600-1335	265	100.00
OP-07	07	03	1975-1350	625	042.85
OP-08	04	02	1353-1078	275	050.00
OP-11	06	01	1400-925	475	016.66
OP-12	08	05	1725-600	1125	062.50
OP-13	07	04	1250-925	325	057.14
OP-15	06	03	978-325	653	050.00
OP-16	05	01	1650-825	825	020.00
Total	63	36			55.33%

Table 4. Band sharing frequency within and between Holstein-Friesian and Jersey Breed

Primer Code	Band Sharing Frequency (BSF)		BSF between Holstein-Friesian and Jersey Breed
	Within Holstein-Friesian	within Jersey	
OP-01	0.83	0.78	0.21
OP-03	0.91	0.90	0.34
OP-04	0.84	0.82	0.19
OP-07	0.94	0.89	0.28
OP-08	0.86	0.92	0.14
OP-11	0.78	0.85	0.09
OP-12	0.92	0.94	0.29
OP-13	0.67	0.86	0.08
OP-15	1.00	0.96	0.59
OP-16	0.95	0.88	0.24
The overall: 0.90 ± 0.03		0.88 ±	0.30± 0.06

Band sharing frequency: the average band sharing frequency within and between breeds in Holstein and Jersey are given in Table (4). The frequency varied in Holstein-Friesian from 1.00 – 0.67 with respect primer OP-15 and OP-13, Barwar *et al*. (2008) when they studied on Murrah and Bhadawari buffaloes, they reported similar results in band sharing frequency of Murrahbuffalo were (1.00 to 0.81). While in Jersey, it varied from 0.96 – 0.78 for primer OP-15 and OP-01 respectivly. Similarly band sharing frequency between Holstein-Friesianand Jersey breed varied from 0.59 – 0.08 for the primer OP-15 and OP-13 respectively, Table (4). The pooled aveargе band sharing ferquency within Holstein-Friesian breed was 0.90± 0.03 and Jersey breed was 0.88±0.02. BSF between Holstein-Friesian and Jersey breed was about (0.30 ±0.06), Dakheel *et al*. (2013) when they studied on band sharing frequency between Murrah and Syrti breed, they reported similar results about (0.28±0.2). Genetic distance the genetic calculated for various primers are given in Table (5). All the primers using showed genetic distance. Primer OP-13 showed highest genetic distance (2.21) between two these breeds while the primer OP-15 indicated lowest genetic distance (0.33). The overall average genetic distance was 1.40±0.17between Holstein-Friesianand Jersey breed, same results reported by Dakheel *et al*. (2013), when they showed the genetic distance between Murrah and Syrti breed was (1.41±0.05). No significant genetic distance was found between the breeds with respect to the primers used. Barwar *et al.* (2008) observed the average genetic distance between Murrah and Bhadawari breeds of Indian buffalo using RAPD-PCR were (1.20±0.1).

Table 5. Percentage difference and Genetic Distance between Holstein-Friesian and Jersey Breed

Primers	PercentageDifference between Murrah & Surti	Genetic distance between Murrah & Surti
OP-01	41.12	1.50
OP-03	66.98	1.25
OP-04	58.32	1.34
OP-07	22.94	1.87
OP-08	82.30	0.93
OP-11	38.50	1.60
OP-12	79.58	0.96
OP-13	19.81	2.21
OP- 15	90.69	0.33
OP-16	43.95	1.40
Mean average:	54.41 ± 3.82	1.40±0.17

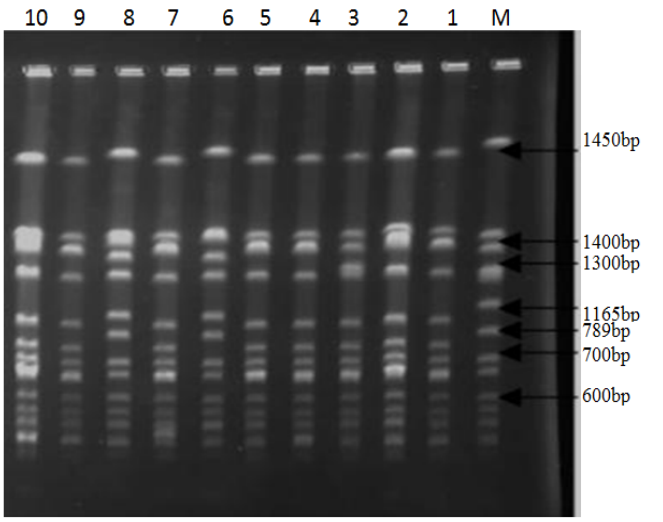

Fig. 1. Holstein-Friesianbreed under UV illuminator by using RAPD technique and primer OP- 11. M= 100 ladder

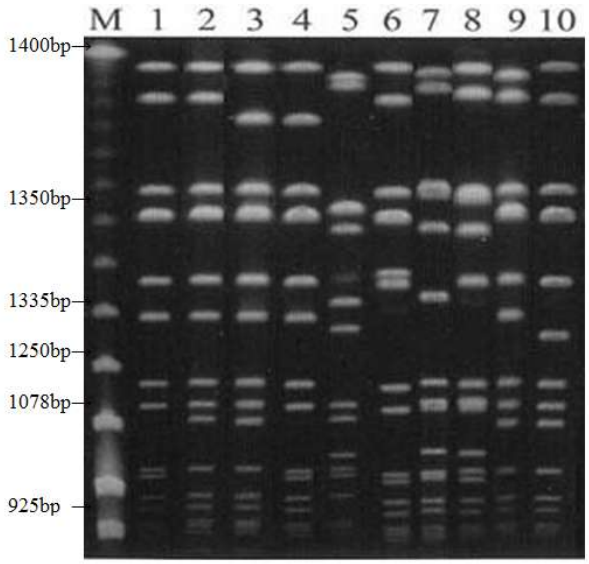

Fig. 1. Jersey breed under UV illuminator by using RAPD technique and primer OP- 11. M= 100 ladder

The MAPD value was calculated as a measure of inter breed divergence from the RAPD fingerprints obtained with 10 primers. The average percentage difference (APD) estimated for all the primers is percentage in Table (5) the highest APD value 90.69 between these two breeds obtained with primer OP-15 and lowest value of 19.81 with primer OP-13. The MAPD between these two breeds was estimated to be 54.41±3.82%. The values indicate high genetic variation between these two breeds. Fadhil *et al*. (2013) also reported MAPD value was about (67.10±0.15) between Holstein-Friesianand Jersey breed by using different set of primers.

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