Genetic Distance Study among Deoni Breed of Cattle Using Random Amplified DNA Markers

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ABSTRACT : Random amplified polymorphic DNA (RAPD) analysis was done with 19 oligonucleotide primers to study genetic similarities and divergence among different types of Deoni breed of cattle viz., Balankya, Wannera and Waghya. Six random primers produced low to high numbers of polymorphic bands between pooled DNA of different Deoni types. Of the 48 RAPD markers obtained 33 were common to all Deoni types, 3 were individual specific and 12 were polymorphic for different Deoni types. The mean average percentage difference values among Deoni types showed that Balankya and Wannera had less genetic divergence when compared to Waghya. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 315-319)***

Key Words : Deoni, Genetic Similarities and Divergence, RAPD, DNA Fingerprints

INTRODUCTION

Deoni breed of cattle is one of the 26 recognized cattle breeds (*Bos indicus*) of India. The breeding tract of Deoni cattle includes mainly the districts of Bidar (Karnataka state), Latur (Maharashtra state) and neighbouring areas in India that lies between 17° 30' and 18° 55' north and 76° 30' and 77° 55' east. The Deoni breed has a history of over 300 years. Genetically, the Deoni breed was evolved through the crossbreeding of the Gir cattle of the Kathiawar region of Gujarat with the Dangi breeds of Marathawada and local desi cattle of Nizam state from Bidar and Osmanbad (Joshi and Phillips, 1953). Gir cattle is known for it's milk production, while Dangi, a breed native to Nasik in Maharashtra is known for being very hardy and possessing good stamina. The Deoni breed, over the years, has evolved into three morphological types that are distinguished by their body colour patterns. The Deoni types evolved are Balankya, animals with complete white body coat and without any spot on the body; Wannera, animals with white body and black shades on sides of the face and Waghya, animals with white and black shades/spot/patches scattered all over the body. The emergence of these morphological types necessitated studying the genetic distances among these Deoni types.

Random amplified polymorphic DNA markers are based on amplification of DNA through the PCR technique using random sequence primers (Williams et al., 1990). The number and size of amplified products depend on the complementarity of sequences of the particular primer and template DNA. A single base change in the primer-binding site in the genome may prevent amplification by introducing a mismatch at just one end of a DNA segment. Structural alterations such as deletion of priming sites, insertion of sites that render priming sites too distant to support amplification and insertions that change the size of DNA segments without preventing their amplification result in RAPD polymorphisms. RAPD fingerprinting has been widely used for breed characterization in cattle (Gwakisa et al., 1994, Nagaraja, 1998 and Ramesha et al., 2001).

The present study was conducted with the objectives of determining genetic similarities and divergence among different Deoni types and to identify suitable markers unique to each type.

MATERIALS AND METHODS

DNA samples

Genomic DNA was islolated by the high salt method as described by Montgomery and Sise (1990) from peripheral blood of 30 male and 40 female Deoni cattle. The pooled DNA samples were prepared for each Deoni type and sex by adding equal quantities of DNA from all the animals. Minimum of 12 blood samples were collected per Deoni type.

Primers

A total of 19 arbitrary oligonucleotide primers obtained from Bangalore Genei (P) Ltd. India, were used singly for amplification of Deoni type and sex specific DNA pools. Those primers, which could discriminate the DNA pools, were investigated further on 12 random individual DNA samples that were constituent of the pooled DNA of each Deoni type to examine the frequencies of amplified fragments in all the individuals constituting each of the DNA pool.

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PCR amplification

The amplification reactions were carried out in 0.2 ml microcentrifuge tubes using a programmable thermal cycler (Eppendof Mastercycler, Germany). Each 30 µl reaction mixture comprised of 20 ng of template DNA, one µl of primer (40 p. mol/µl), 200 mM of each dNTP (Bangalore Genei), 0.75 unit of Taq DNA polymerase (Bangalore Genei), and 1×reaction buffer (Bangalore Genei). The 1× reaction buffer consisted of 10 mM Tris HCl (pH 8.3), 50 mM of KCl and 0.1 per cent Triton X-100. The content was mixed thoroughly and centrifuged for five seconds at 5,000 rpm. A drop of mineral oil was overlaid to avoid evaporation. A PCR programme with initial denaturation at 95°C for 2 min. and 39 cycles each consisting denaturation for 45 sec. at 94°C, annealing at 35°C for 1 min and extension for 2 min. at 72°C, was employed for amplification. A final extension at 72°C for 3 min. was also included in the programme.

After completion of the PCR reaction, the PCR products were electrophoresed at 100 volts in 2.5 per cent agarose gel in 1×Tris-borate-EDTA (TBE) buffer containing ethidium bromide (Biorad) at the rate of 8 µl/ 100 ml of 1× TBE buffer. At the end of electrophoresis, the RAPD bands were visualized and documented in a gel documentation system (Gel doc unit 2000, Biorad, USA).

Analysis of RAPD fingerprints

Scoring of RAPD bands was done by using Quantity 1 (Biorad, USA) software programme and was analyzed according to Gwakisa et al. (1994). Only distinct and prominent bands were scored. Comparisons of RAPD fingerprints were made only on samples run on the same gel by using following equations.

Band sharing (BS)

Band sharing (Gwakisa et al., 1994) was calculated as an expression of similarity of RAPD bands by using the formula of Nei and Li (1979).

$BS=2(B_{ab})/(B_a+B_b)$

Where,

B_{ab} is the number of bands shared by individuals a and b.

B_a is the total number of bands for individual a.

B_b is the total number of bands for individual b.

BS values were calculated between individuals of the different Deoni types and also among individuals of the same Deoni type, which constituted the type specific pooled DNA. Average BS value was calculated by dividing the sum of BS values of pair wise comparisons by the total number of pairs compared.

Mean average percentage differences (MAPD)

The dissimilarities between the DNA pools were expressed in the form of MAPD described by Gwakisa et al. (1994) by using the following formulae.

$$\text{Percentage differences (PD)} = \frac{N_{ab}}{N_a+N_b} \times 100$$

$$\text{Average percentage differences (APD)} = \frac{1}{C} \Sigma \, Pd_i$$

$$\text{Mean average percentage difference (MAPD)} = \frac{1}{R} \Sigma \, APD_i$$

Where,

- N_{ab} is the number of fragments that differed between two individuals for a single primer
- N_a is the number of fragments resolved in individual a.
- N_b is the number of fragments resolved in individual b.
- C is the number of interbreed pair wise comparisons
- R is the number of random primers used.

RESULTS AND DISCUSSION

Screening of RAPD primers

Out of 19 random primers, only 8 amplified genomic DNA and of these only six primers (ILO 526, ILO 868, ILO 876, ILO 1065, OPAV 15 and OPAX 19) produced low to high numbers of polymorphic bands between the pooled DNA of different Deoni types. PCR-RAPD analysis of different pooled DNA samples for each Deoni type and sex and individual DNA samples, which were constituents of the pooled DNA samples revealed that the number of RAPD bands amplified in different Deoni types ranged from 5 to 13. Number of RAPD bands per primer in different Deoni types is given in table 1. Size of the PCR products ranged from 0.23 (primer ILO 868) to 2.32 kb (ILO 1065). The total number of RAPD markers obtained from the six primers which produced polymorphic bands was 48, out of which, 33 were common to all Deoni types, three were type specific and 12 were polymorphic for different Deoni types.

Table 1. Number of RAPD bands per primer in different Deoni types

Primers / Deoni types	ILO 526	ILO 868	ILO 876	ILO 1065	OPAX 19	OPAV 15
Balankya	5-7	4 -6	4-7	5-7	3-5	8 -12
Wannera	4-6	4	3-5	9-10	3-4	9-12
Waghya	4-5	2-4	3-6	7-10	4-5	7 -13

Primer ILO 526 produced a moderate number of polymorphic bands (figure 1). The fragment of size 0.80 kb was found in the Deoni type Balankya and type Wannera DNA pools and was absent in the Deoni type Waghya DNA pool. On amplification of DNA of individual animals, the 0.80 kb fragment appeared in 66.67 per cent of individual DNA of Deoni type Balankya and 75 per cent of individual DNA of Deoni type Wannera. A fragment of size 0.77 kb was detected in Deoni breed (pooled). It had earlier been reported to be present in Ongole and Krishnavalley breeds and absent in other South Indian zebu breeds of cattle (Ramesha et al., 2001). Krihnavalley and Ongole like Deoni are dual purpose breeds while the other South Indian zebu breeds are draft animals.

Primer ILO 526 was known for its ability to distinguish between *Bos taurus* and *Bos indicus* cattle of African origin. Earlier workers observed a specific 0.50 kb product amplified only in *Bos indicus* breeds of cattle of African origin and not in *Bos taurus* (Kemp and Teale, 1994). Contrary to their report and similar to the findings in the present study, Nagaraja (1998) and Ramesha et al. (2001) failed to detect the 0.50 kb fragment in *Bos indicus* cattle of Indian origin. One possible explanation could be that *Bos indicus* cattle of African origin and *Bos indicus* cattle of Indian origin were genetically divergent.

Primer ILO 868 was found to produce a high number of polymorphic bands among DNA pools of different types of Deoni cattle but failed to produce any consistent and reliable Deoni type specific fragment (figure 2). A 0.46 kb fragment was detected in the DNA pools of Deoni types Balankya and Waghya while a 0.31 kb fragment was detected in the DNA pools of Deoni types Balankya and Wannera. Fragment of size 0.46 kb was amplified in all the individuals of Balankya type and 83.33 per cent individuals of Waghya type while the fragment of size 0.31 kb was amplified in all the individuals of Wannera type and 83.33 per cent individuals of Balankya type.

Primer ILO 876 produced more polymorphic bands than the other primers used in the present study for the amplification of Deoni DNA. This was also reflected by it having the highest average percentage difference value when compared to the other primers. Though the primer ILO 876 produced a high number of polymorphic bands it failed to produce consistent PCR- RAPD product specific to a particular Deoni type. The amplified product of Deoni type Balankya gave the Deoni type specific fragments of 1.17 kb and 0.37 kb. However, these fragments were not found in all the individuals of Balankya. In the present study, fragment 0.87 kb was amplified in all the individual DNA of Deoni cattle. Nagaraja (1998) had also reported that primer ILO 876 produced a fragment of about similar size in both *Bos indicus* and *Bos taurus* cattle.

Left to right
Lane No.

1	100 bp DNA molecular weight marker
2	Male
3	Female
4	Balankya
5	Wannera
6	Waghya
7	Control
8	DNA molecular weight marker (phi×174 DNA Hae III digest)

Figure 1. Amplification by random primer ILO 526 of Deoni cattle DNA pooled by type and sex wise.

Left to right
Lane No.

1	Male
2	Female
3	Balankya
4	Wannera
5	Waghya
6	Control
7	100 bp DNA molecular weight marker

Figure 2. Amplification by random primer ILO 868 of Deoni cattle DNA pooled by type and sex wise.

Table 2. Mean average percentage differences among different Deoni types

Deoni types	Balankya	Wannera	Waghya
Balankya	- (0.875)	11.54 ±2.98 (0.885)	13.26±3.53 (0.844)
Wannera	-	- (0.901)	13.76±3.00 (0.867)
Waghya	-	-	- (0.835)

Figures in parenthesis denote average band sharing values between and within Deoni types.

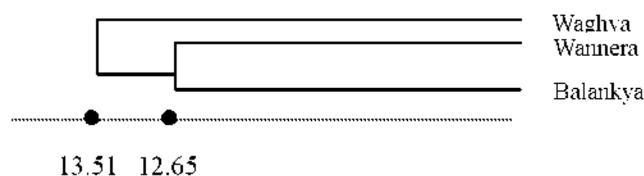

Figure 3. Dendrogram of relationship among three Deoni types.

Primer ILO 1065 produced a moderate number of bands from DNA pools of different types of Deoni cattle. This primer was reported to produce a *Bos indicus* (African origin) male specific fragment of 1.13 kb size (Teale et al., 1995). However, samples pooled by sex and individual samples of DNA of *Bos indicus* (Indian origin) tested using this primer failed to amplify the 1.13 kb fragment (Nagaraja, 1998). In the present study ILO 1065 amplified a specific fragment 0.96 kb in all DNA from individual male Deoni suggesting that this primer was suitable for differentiating male Deoni from female Deoni. It also produced a 0.56 kb fragment in all individual DNA of Deoni types Wannera and Waghya which was not amplified in the DNA of the Balankya type implying that ILO 1065 also provide a convenient means of differentiating Balankya from the other Deoni types.

Compared to the primers ILO 526, ILO 868, ILO 876 and ILO 1065, the primers OPAV 15 and OPAX 19 have been used to a lesser extent in RAPD analysis for breed characterization. In the present study both OPAV 15 and OPAX 19 produced low to moderate numbers of polymorphic bands. Both the primers detected no (Waghya and reliable type specific fragment. However in terms of the numbers of bands produced, OPAV 15 produced the highest number of bands (13) with band sizes ranging from 0.26 kb to 1.45 kb.

Band sharing between and within Deoni types

Genetic divergence between the different types of the Deoni breed of cattle was calculated in terms of average band sharing (BS) and mean average percentage difference (MAPD) based on pooled results of all the primers. The average BS values among the different Deoni types for pooled DNA samples are presented along with MAPD values in table 2. A dendrogram of relationships among the three Deoni types estimated by the Distance Wagner procedure (Farris, 1972) is depicted in figure 3. The mean average percentage difference between Deoni types Balankya and Wannera, Balankya and Waghya, Wannera and Waghya were 11.54±2.98, 13.26±3.53 and 13.76±3.00. As expected, the MAPD values between the Deoni types were lower than those reported by Nagaraja (1998) and Ramesha et al. (2001) between different breeds. Though there was no statistical difference ($p \leq 0.05$) between the MAPD values among the different Deoni types. Deoni types Balankya and Wannera had less genetic divergence between them when compared to Deoni type Waghya.

CONCLUSIONS

The RAPD technique can be used to differentiate between Deoni types and sex. Primer ILO 1065 can differentiate male and female Deoni cattle. The other primers produced bands, which showed polymorphisms among the different Deoni types, but failed to give any consistent and reliable type specific RAPD marker. The band sharing and MAPD indicated that there is less genetic divergence between Balankya and Wannera compared to Waghya. No significant difference among the three types of Deoni cattle was observed at the molecular level suggesting that interbreeding among these divergent types is a possibility for further genetic improvement of the breed.

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