

Full Length Research Paper

Genetic variability of Indian yaks using random amplified polymorphic DNA markers

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Indian yaks are categorized into common yaks, bisonian yaks and bareback yaks. Genetic similarities and divergence among them was analyzed by random amplified polymorphic DNA (RAPD) technique using ten decamer oligonucleotide primers. Only five primers (ILO 526, OPAV 15, ILO 1127, ILO 1065 and ILO 876) out of the ten primers tried produced consistent polymorphic fingerprints. Of the 76 fingerprints produced, 49 were present in all types, 21 were individual specific and 6 were polymorphic for different types. The pair wise comparison studied for different primers indicated the average bands sharing ranged from 78% in bareback to 88% in common type. There was no significant difference ($P \leq 0.05\%$) between the mean average percentage (MAPD) values observed between Indian yaks.

Key words: Yak types, RAPD, DNA fingerprints.

INTRODUCTION

Yak is a unique bovine species of economical importance in high hill and snow bound areas. It provides milk, meat, hair/wool, transport, draught and fuel to the high landers. The domesticated yaks (*Poephagus grunniens* or *Bos grunniens*), a native of Tibetan plateau, are originated from wild yaks (*Poephagus mutus przewalski*). In India, yaks are mainly distributed in the Himalayan states of Himachal Pradesh, Jammu and Kashmir, Sikkim and Arunachal Pradesh. Pal et al. (1994) categorized Indian yaks into three types, common yaks, bisonian yaks and bareback yaks and their respective population was 56.2, 29.8 and 14.0%. The common yaks are comparatively smaller in size, the appearance of the head and body conformation are like cattle. The bisonian type yaks are hefty animals with compact head connected by strong and short neck to the main trunk. The bareback yaks are

bodily rectangular with short hairs on rump and wither. The rosette of hairs hangs down the belly line and almost touching the ground. Characterization and evaluation of genetic difference among these types is necessary for effective and meaningful conservation of Indian yaks.

Random amplified polymorphic DNA (RAPD) markers are based on amplification of DNA through the polymerase chain reaction amplification (PCR) technique using random sequence primers (Williams et al., 1990). The number and size of amplified product depends on the complementary sequence of the particular primer and template DNA. RAPD finger printing has been widely used for breed characterization in cattle (Gwakisa et al., 1994; Appannavar et al., 2002) and buffalo (Shashidhara et al., 2004). RAPD fingerprints have also been used to study the genetic variation in Chinese five yak breeds (Yuping et al., 2004). However, no information is available on the genetic similarities and divergence among the yak types of India. Hence, the present study was conducted with the objectives of determining genetic similarities and divergence among different Indian yak types and to identify RAPD fingerprints unique to each

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type.

MATERIALS AND METHODS

Experimental animals and isolation of DNA

Genomic DNA was isolated by the high salt method as described by Montgomery and Sise (1990) from peripheral blood of 35 female and 40 male yaks. Among the 35 females used in the study, 15 were common type and 10 each belonged to bisonian and bareback types. The 40 males included 15 each from common and bisonian type and 10 belonged to bareback type. The pooled DNA samples were prepared for each yak type and sex by adding equal quantities of DNA from all the yaks. Minimum of 10 blood samples were collected per yak type.

Primers

Ten arbitrary oligonucleotide primers obtained from Bangalore Genei (p) Ltd., India were used singly for amplification of DNA from Indian yak type and sex specific DNA pools. Those primers, which could discriminate the DNA pools, were investigated further on a minimum of ten random individual DNA samples of each yak type that were constituent of the pooled DNA.

PCR amplification

The amplification reactions were carried out in 0.2 ml micro centrifuge tubes using a programmable thermal cycler (Eppendorf Mastercycler, Germany). Each 30 μ l reaction mixture comprised of template DNA 20 ng, Primer 1 μ l (40 p. mol/ μ l), dNTP 200 μ M, Taq DNA polymerase 0.75 unit, 1X reaction buffer which comprises 10 mM Tris HCl (pH 8.3), 50 mM of KCl and 0.1% Triton X-100. The content was mixed thoroughly and centrifuged for 5 s at 5000 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 s 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. The PCR products were electrophoresed at 100 volts in 2.5% agarose gel in 1x Tris- borate-EDTA (TBE) buffer containing ethidium bromide at the rate of 8 μ l/100 ml of 1X TBE buffer. After electrophoresis, the RAPD bands were visualized and documented in gel documentation system (Gel Doc unit 2000, Biorad, USA).

Analysis of RAPD fingerprints

Only reproducible bands were scored for the presence or absence ignoring the intensity of band. Scoring of RAPD bands was done by using Quantity 1 (Biorad, USA) software programme and was analyzed according to Gwakisa et al. (1994). Comparison of RAPD fingerprints were made only on samples run on the same gel using the following equations.

Band sharing (BS)

Band sharing 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 and B_b is the total number of bands for individual b.

BS values were calculated between individuals of the different yak types and also among individuals of the same yak 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 difference (MAPD)

MAPD values were calculated as a measure of the intertype genetic divergence and were calculate as described by Gwakisa et al. (1994) by using the following formulae.

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

$$\text{Average percentage differences (APD)} = \left(\sum_{i=1}^C PD_i \right) / C$$

$$\text{Mean average percentage difference} = \left(\sum_{i=1}^R APD_i \right) / R$$

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

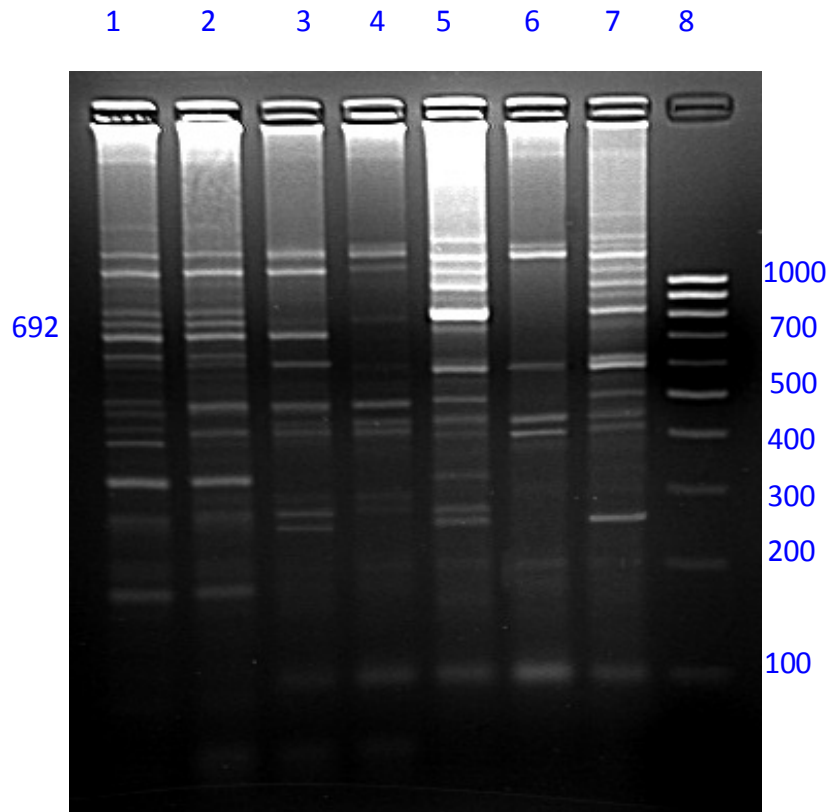
RESULTS

Ten random decamer primers were used for amplification of pooled genomic DNA of common, bisonian and bareback types of yaks, of which only five primers (ILO 526, OPAV 15, ILO 1127, ILO 1065 and ILO 876) produced low to high numbers of polymorphic bands. The number of RAPD bands observed was in the range of 2 to 12 with molecular size ranging from 92 (ILO526) to 3187 bp (ILO876). Only distinct bands were scored for further analysis. Number of RAPD bands per primer in different yak type is given in Table 1. The total number of RAPD markers obtained from the five primers was 76 out of which 49 of these were present in all types, 21 were individual specific and six were polymorphic for different types.

Primer OPAV15 was found to produce a high number of polymorphic bands (Figure 1) among DNA pools of different types of yaks but failed to produce any consistent and reliable type specific fragment. A band of 692 bp was observed in all the males irrespective of type, while only 12% of females showed the same. Primer ILO526 produced moderate number of bands. The prominent fragment of size 688 bp was observed in all the three types of either sex. Another fragment of size

Table 1. Number of RAPD fingerprints per primer in yak types.

Primer yak type	ILO 526	OPAV 15	ILO 1127	ILO 1065	ILO 876
Bisonian	4-6	6-9	5-7	5-7	5-6
Common	4-8	9-10	4-6	5-6	5-10
Bareback	6-8	7-9	4-6	5-6	7-8

**Figure 1.** Amplification of type and sex wise pooled yak DNA by random primer OPAV15. Lane 1, Common type male; lane 2, bisonian type male; lane 3, bareback type male; lane 4, bareback type female; lane 5, yak over all-irrespective of sex and type; lane 6, bisonian type female; lane 7, common type female; lane 8, 100 bp ladder DNA marker.

1402 bp was present in common and bareback types but not in bisonian type. Another fragment of about 860 bp was observed only in females of all the three types. The RAPD primer ILO1127 has successfully amplified yak DNA and produced moderate number bands. Primer ILO1065 also produced moderate number of fingerprints. ILO876 produced moderate to high number of bands.

Band sharing between and within yak types

Genetic divergence between the different types of Indian

yaks were calculated in terms of average band sharing (BS) and mean average percentage difference (MAPD) based on pooled result of all primers are presented in Table 2. The pairwise comparison studied for different primers indicated that, the average bands sharing ranged from 78% in bareback to 88% in common type. The mean average percentage difference (MAPD) between common and bisonian, common and bareback, bisonian and bareback were 12.26 ± 3.52 , 14.89 ± 2.99 and $14.73 + 2.83$. There was no significant difference ($P \leq 0.05\%$) between the MAPD values among common, bisonian and bareback types.

Table 2. Mean average percentage differences among different yak types.

Yak type	Common	Bisonian	Bareback
Common	(0.884)	12.26±3.25 (0.871)	14.89±2.99 (0.833)
Bisonian	-	(0.862)	14.73±2.83 (0.835)
Bareback	-	-	(0.781)

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

DISCUSSION

In the present study, OPAV15 was found to be highly polymorphic and ILO 1065 produced moderate number of bands. Similar reports have been reported earlier in cattle by Appannavar et al. (2001). There was no significant difference ($P \leq 0.05\%$) between the MAPD values among common, bisonian and bareback types. Yuping Xiao (2004) reported the genetic diversity in Datong yak, Jiulong yak, Tianzhu yak, Maiwa yak (Hongyuan) and Maiwa Yak (Ruoergali) of China as 0.207, 0.422, 0.178, 0.207 and 0.369, respectively. The genetic diversity among Indian yaks observed in the present study is lower than the Chinese yaks. The study indicated that, all the three types of Indian yaks are genetically similar. There are no earlier reports on these yak types to compare or contrast the present results. The study implies that, there is no need to conserve different types of yaks separately unless unique genes/gene sequences with known utility are identified using recent molecular genetic tools.

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