Assessment of Bandsharing Values in RAPD-PCR Analysis of Dwarf Cattle of Kerala

P. Suprabha*, K. Anilkumar, T. V. Aravindakshan and K. V. Raghunandanan

Centre for Advanced Studies in Animal Genetics and Breeding, College of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur-680651, Kerala, India

ABSTRACT : Random amplified polymorphic DNA (RAPD-PCR) analysis of 56 animals of four different genetic groups of dwarf cattle in Kerala was done as a single step analysis. Bandsharing (BS) values were calculated for animals of each group and between groups as an analytical tool to find out genetic variation among animals. The different factors affecting BS values were estimated using Harvey's Least squares analysis. The effects of genetic group, Guanine-cytosine (GC) content of primer and gel on BS values were found significant. Bandsharing values of Kasargode-Highrange dwarf animals were significantly different from Vechur, Vatakara and their combinations. The Vechur, Vatakara and Vechur-Vatakara combinations were found to be more uniform (high BS value) compared with other combinations. The bandsharing value was lowest with primers of GC content 90% and highest with 80% GC content. The effect of gel on BS value points to the need of adjustments of gel factor for calculation of BS values. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1217-1220)***

Key Words : Random Amplified Polymorphic DNA Marker, Bandsharing Value, Dwarf Cattle

INTRODUCTION

Random amplified polymorphic DNA markers (RAPD) were first described by Williams et al. (1990). The technique is simple, fast and relatively cheaper, permitting rapid generation of almost unlimited number of polymorphisms. It uses random oligonucleotide primers at low annealing temperature for amplification. The polymorphisms are due to the difference in spacing between primer binding sites or due to point mutations, which allow or abolish primer binding. The greatest advantage of this technique is its ability to obtain DNA polymorphisms without prior knowledge of genomic sequence. As the marker scans the entire genome, it is used in characterization studies of animals and plants. Bandsharing (BS) values were calculated from the RAPD fingerprints as an analytical measure to estimate the genetic divergence of animals, which is very well needed for characterization of animals. One of the disadvantages of RAPD-PCR technique is the influence of different factors in band formation, which will affect bandsharing values. Standardization of the amplification conditions needed for RAPD-PCR analysis have been done for repeatability. The present study was an investigation towards the factors influencing BS value in RAPD-PCR analysis.

MATERIALS AND METHODS

Genetic divergence in dwarf cattle of Kerala was studied using RAPD-PCR technique. Animals belonging to four native dwarf cattle groups of Kerala viz. Vechur, Vatakara, Highrange dwarf and Kasargode formed the subjects of the study. Ten oligonucleotide primers were used for individual RAPD-PCR analysis of DNA samples, which were extracted by standard phenol: chloroform isolation procedure. PCR amplifications were carried out by single primer method (Gwakisa et al., 1994).

The PCR reactions were carried out in 0.2 ml thin wall PCR reaction tubes using a programmable thermal cycler (MJ Research Inc., USA). Twenty microlitre reaction was standardized comprising 25 ng of template DNA, 5 pM primer, 200 μM each dNTPs, 0.5 unit *Taq* DNA polymerase, 1.5 mM $MgCl_2$ and 1× PCR buffer. The PCR amplification condition was standardised as follows. After an initial denaturation of 94°C for 1 min, a 35 cycle reaction was performed, with each cycle having 1 min denaturation (94°C), 1 min annealing (38°C) and 1 min extension (72°C). This was followed by a final extension for 10 min at 72°C. The PCR products were electrophoresed at 100 V in 2% agarose gel containing ethidium bromide. The banding patterns were visualized under a UV transilluminator and documented in gel documentation system (Biorad Laboratories, USA).

The RAPD-PCR products were run on 2% agarose gel in two rows as upper and lower. Thirty samples were loaded in each row. The polymorphic patterns produced by RAPD primers were identified as presence or absence of bands. The average Bandsharing value for each primer and for 10 primers were estimated as given by Gwakisa et al. (1994). The bandsharing was calculated by pair wise comparison method, as an expression of similarity of RAPD fingerprints

* Corresponding Author: P. Suprabha. Tel: +91-0471-2321633, Fax: + , E-mail: drsuprabha@rediffmail.com
Received September 22, 2004; Accepted March 22, 2005

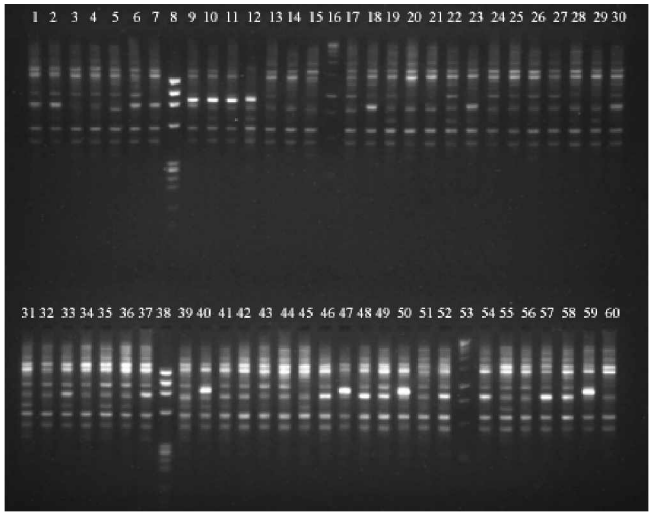

Figure 1. RAPD profile of individual DNA samples with primer OPA 18. Lanes: 1-7 and 31-37: Vechur cattle. 9-15 and 39-45: Highrange dwarf cattle. 17-23 and 46-52 Vatakara cattle. 24-30 and 54-60: Kasargode cattle. 8 and 38 (M_1): Φ×174 (marker) 16 and 53 (M_2): 1 kb* DNA ladder (marker). * kb = kilo basepair.

of animals within or between genetic groups (Dunnington et al., 1990; Gwakisa et al., 1994; Aravindakshan and Nainar, 1998; Islam et al., 2005) using the formula.

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

Where, B_{ab} is the number of bands shared by individual 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.

The different factors affecting bandsharing values such as genetic group, GC content of primer and the different combinations of comparison of gel were analysed using least squares analysis as described by Harvey (1986). The mixed model least squares and maximum likelihood computer programme PC-1 was used. The following mathematical model was constructed.

$$(BS)\ Y_{ijk} = \mu+GC_i+GEL_j+GG_k+e_{ijk}$$

μ = Overall mean.

GC_i = Effect of GC content of primers on bandsharing value

GEL_j = Effect of samples compared in upper row, lower row and both upper and lower row of gel on bandsharing value.

GG_k = Effect of genetic groups on bandsharing value.

e_{ijk} = Error.

$(BS)\ Y_{ijk}$ = Bandsharing value made by pair wise comparison of i^{th} GC content of primer, j^{th} gel, k^{th} genetic group.

Ten combinations of genetic groups [Vechur and Vechur (VV), Vechur and Highrange dwarf (VH), Vechur and Vatakara (VD), Vechur and Kasargode (VK), Highrange dwarf and Highrange dwarf (HH), Highrange dwarf and Vatakara (HD), Highrange dwarf and Kasargode (HK), Vatakara and Vatakara (DD), Vatakara and Kasargode (DK) and Kasargode and Kasargode (KK)], four different classes of primers based on GC content [60%, 70%, 80% and 90%] and the three different combinations of comparisons of gel [upper, lower and combination] were analysed for finding their effect on bandsharing value.

RESULTS AND DISCUSSION

The RAPD-PCR conditions to get reproducible banding pattern varies between laboratories (Penner et al., 1993; Rothuizen and Wolferen, 1994; Ambady et al., 1996; Parejo et al., 1997; Yoon and Kim, 2004). Standardization of RAPD-PCR conditions was done under this study so that reproducible fingerprints were obtained. The various levels of variables that were best suited were identified after conducting a series of tests. Ten random primers were analyzed on individual DNA samples. Different primers produced different RAPD profiles. Figure 1 represents the RAPD profile of animals when amplified with single primer, OPA 18. RAPD fingerprints obtained with individual animals with different primers were used for the calculation of BS values. The bandsharing values within and between four genetic groups were calculated under this study as an indicator of genetic similarities. All possible 15,400 pair-wise combinations of different animals were made for ten primers and the average BS value for each comparison of genetic groups were estimated. Pairwise comparison and band sharing value estimates were used as given by Gwakisa et al. (1994), Mohd-Azmi et al. (2000); Ramesha et al. (2002); Yoon and Park (2002) and Saifi et al. (2004).

In RAPD analysis, minute variations in different factors can affect the product profile. The various variables affecting the bandsharing values were taken into account on least square analysis of variance. The effects of genetic group, GC content of primer and the effect of gel were studied using Harvey's Least squares analysis, Model-1. The least square means for these variables are given in Table 1 and the least squares analysis of variance is presented in Table 2. The effects of GC content of primer, genetic group and the gel on bandsharing values were found significant ($p<0.01$).

Ten least square means of BS value obtained from comparisons of animals within and between genetic groups, Vechur-Vechur (VV), Vechur-Highrange dwarf (VH), Vechur-Vatakara (VD), Vechur-Kasargode (VK), Highrange dwarf-Highrange dwarf (HH), Highrange dwarf-Vatakara (HD), Highrange dwarf-Kasargode (HK), Vatakara-Vatakara (DD), Vatakara-Kasargode (DK) and Kasargode- Kasargode

Table 1. Least squares means of band sharing values for different genetic groups, GC content of primer and gel in RAPD-PCR analysis

Factors (variable)	Level	BS value least square mean±SE
GC content of primer	60%	0.79±0.00[a]
	70%	0.85±0.00[b]
	80%	0.91±0.00[c]
	90%	0.71±0.00[d]
Genetic group (combinations)	VV (900)*	0.83±0.00[b]
	VH (1,960)	0.82±0.01[c]
	VD (2,100)	0.83±0.00[b]
	VK (1,830)	0.81±0.00[c]
	HH (910)*	0.81±0.00[c]
	HD (1,960)	0.82±0.01[c]
	HK (1,960)	0.80±0.00[a]
	DD (910)*	0.83±0.00[b]
	DK (1,960)	0.81±0.01[c]
	KK (910)*	0.80±0.00[c]
Gel	Upper	0.84±0.00[a]
	Lower	0.82±0.00[b]
	Combination	0.78±0.00[c]

The least square means with same superscript within the same variable does not vary significantly ($p \leq 0.01$).

* V: vechur; H: highrange dwarf; D: vatakara; K: kasargode.

(KK) are presented in Table 1. The least square mean for BS values of HK (0.80±0.00) was significantly different from BS values of that of Vechur, Vatakara groups and their combination (VV, DD and VD). The significantly low value of bandsharing for HK suggests that Highrange dwarf and Kasargode cattle are more divergent compared to Vechur and Vatakara cattle. The within group BS values of Vechur and Vatakara animals (0.83±0.00) are comparatively higher than Kasargode (0.80±0.00) and Highrange dwarf animals (0.81±0.00), indicating more within group homogeneity in Vechur and Vatakara animals. The between group BS values did not differ significantly for other comparisons; VH (0.82±0.01), VK (0.81±0.00), HD (0.82±0.01), DK (0.81± 0.01).

The variations of band sharing values in comparisons of upper gel to upper gel (Upper), lower gel to lower gel (Lower) and upper gel to lower gel (Combination) were analyzed. A significant effect was observed for gel effect on BS value. The upper gel had the highest BS values (0.84±0.00) followed by the lower gel (0.82±0.00) and the combination had the least value (0.78±0.00). These indicate that the comparison of between gel bandsharing values may not yield exactly the same results as that of a single gel. Gwakisa et al. (1994) and Aravindakshan and Nainar (1998) used samples run on same gel for comparison. From this, it was clear that between gel comparisons had considerable effect on BS values. Hence the effect of gel has to be adjusted before analysis of BS values.

Table 2. Least squares analysis of variance for the effects of GC content of primer, genetic group and gel on bandsharing

Source	Degrees of freedom	Mean sum of squares	F value	p value
GC content of primer	3	13.54	499.06**	0.00
Genetic group	9	0.20	7.44**	0.00
Gel	2	4.53	166.88**	0.00
Error	15,385	0.03		
Total	15,400			

** $p \leq 0.01$.

Of the four different classes of primers used based on the GC content, the one with 90 percent GC content yielded significantly lowest bandsharing value (0.71) followed by primers with 60 percent (0.79), 70 percent (0.85) and 80 percent (0.91) GC content respectively. This information emphasizes the role of primers and their GC content on BS values. Williams et al. (1990) reported that 10-base random primers with GC content of 60-90 percent was suitable for amplification of genomic DNA segments for studying the polymorphisms between different individuals.

CONCLUSION

The technique of RAPD-PCR in animals has been standardized and the genetic diversity among native cattle groups was found based on Bandsharing values. The different factors affecting bandsharing value were assessed using Harvey's Least squares analysis of variance. The Highrange dwarf and Kasargode animals differed significantly from Vechur, Vatakara and their combinations. The comparisons of animals of VV, VD and DD showed more uniformity. Hence Vechur and Vatakara animals are found as more similar compared with Highrange dwarf and Kasargode animals. These combinations were different from VH, HH, DK, VK and HD combinations. The effects of GC content of primer, genetic group and the gel on BS values were significant. Adjustments for GC content of primer and gel are needed before assessing BS values in RAPD-PCR analysis ($p<0.01$).

REFERENCES

Ambady, S., C. M. Carpio and F. A. Ponce-de-Leon. 1996. Optimization of RAPD-PCR conditions in cattle. Anim. Biotech. 7(2):99-112.

Aravindakshan, T. V. and A. M. Nainar. 1998. Genetic variation in cattle and buffalo breeds analysed by RAPD markers. Indian J. Dairy Sci. 51(6):368-374.

Bowditch, B. M., D. G. Albright, J. G. K. Williams and M. J. Braun. 1993. Use of randomly amplified polymorphic DNA markers in comparative genome analysis. Methods Enzymol. 224:294-309.

Dunnington, E. A., O. Gal, P. B. Siegel, A. Haberfeld, A. Cahaner, U. Lavi, Y. Plotsky and J. Hillel. 1991. Deoxyribonucleic acid fingerprint comparisons between selected populations of

chicken. Poult. Sci. 70(3):463-467.

Gwakisa, P. S., S. J. Kemp and A. J. Teale. 1994. Characterization of zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. Anim. Genet. 25:89-94.

Islam, M. S., A. S. I. Ahemed, M. S. Azam and M. S. Alam. 2005. Genetic analysis of three river populations of Catla Catla (HAMILTON) using Randomly amplified Polymorphic DNA markers. Asian-Aust. J. Anim. Sci. 18(4):453-457.

Harvey, W. R. 1986. Mixed model Least-Squares and Maximum Likelihood Computer Programme, PC version (PC-1) LSMLMW with Parncard.

Mohd-Azmi, M. L., A. S. Ali and W. K. Kheng. 2000. DNA fingerprinting of red jungle fowl, village chicken and broilers. Asian-Aust. J. Anim. Sci. 13(8):1040-1043.

Parejo, J. C., M. E. Sansinforiano, A. Rabasco, M. Martinez-Trancon, J. L. Fernandez-Garcia and J. A. Padilla. 1997. Optimization of the random amplified polymorphic DNA technique in white Caceres cattle. Archivos-de Zootecnia 46:279-289.

Penner, G. A., A. Bush, R. Wise, W. Kim, L. Domier, K. Kasha, A. Laroche, G. Scoles, S. J. Molnar and G. Fedak. 1993. Reproducibility of Random Amplified Polymorphic DNA (RAPD) analysis among Laboratories. PCR Methods and Applications 2:341-345.

Ramesha, K. P., T. Saravanan, M. K. Rao, M. M. Appannavar and A. O. Reddy. 2002. Genetic distance among South Indian breeds of zebu cattle using random amplified DNA markers. Asian-Aust. J. Anim. Sci. 15(3):309-314.

Rothuizen, J. and M. V. Wolferen. 1994. Randomly amplified DNA polymorphisms in dogs are reproducible and display Mendelian transmission. Anim. Genet. 25:13-18.

Saifi, H. W., B. Bharat, S. Kumar, P. Kumar, B. N. Patra and S. Arjava. 2004. Genetic identity between Bhadawari and Murrah breeds of Indian buffaloes (*Bubalus bubalis*) using RAPD-PCR. Asian-Aust. J. Anim. Sci. 17(5):603-607.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18(22):6531-6535.

Yoon, J. and Kim Jong-Yeon. 2004. Genetic differences within and between populations of Korean cat fish (*S. asotus*) and Bull head (*P. fulvidraco*) analysed by RAPD-PCR. Asian-Aust. J. Anim. Sci. 17(8):1053-1061.

Yoon, J. and H. Park. 2002. Genetic similarity and variation in the cultured and wild crucian carp (*Carassius carassius*) estimated with random amplified polymorphic DNA. Asian-Aust. J. Anim. Sci. 15(4):470-476.