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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Genetic association between the black dotted nose and the yellow coloured nose in Hanwoo using RAPD marker

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Abstract

This study was conducted to evaluate the genetic distances and specific DNA makers by the randomly amplified polymorphic DNA (RAPD)-PCR method for the black-dotted nose and the normal yellow nose in Hanwoo, the Korea native cattle. Genomic DNA was extracted from blood and nose tissues from Hanwoo after they were slaughtered. The extracted DNA was observed by nano-spectrometer. RAPD analysis was performed using 5 different primers. Statistical analysis was made for the estimation of the genetic distance among the cattle's and the cluster tree was drawn by using MEGA 5.05 software. Genetic relations among them were determined by RAPD analysis. The polymorphic bands were observed 67% and the rest of 33% was monomorpic. The largest genetic distance (0.256) was found between the normal yellow nose and the black-dotted nose by UPGMAP method and the closest distance was observed between the black-dotted noses in Hanwoo as expected.

Keywords: RAPD; UPGMAP; black-dotted nose; cattle; Hanwoo

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Introduction

The phylogenic classification of cattle's leads to *Bos Taurus* from Europe, *Bos indicus* from India, and the mixture of the two (MacHugh et al., 1997; Bradley et al., 1998). Korea native cattle's have different colour patterns; yellow, black, and black mixed with yellow on the body as reported by Choi (2009) and Kim et al. (2011). Since the crossbreeding of Hanwoo with other imported breeds started in early 1980's, some mixed colours have often been detected in the registered purebred Hanwoo (Choi, 2009). In 1974, according to the report of National Agricultural Cooperative Federation, they found 91.0% of the yellow-brown, 0.3% of the mixed colour on body, and partially mixed colour being 3.0%, which indicates that body colours are quite homogeneously expressed in Hanwoo. In the report of year 2012 from the Ministry of Agriculture, Fishery and Food, Korea, other than standard colours or colour patterns are recommended to be excluded from the population. However, it has not been easy to implement the elimination of non-standard colours due to frequent expression of non-standard patterns from the phenotypically normal parents in Hanwoo. Lee et al. (2002) and Lee et al. (2011) reported the colour patterns on nose were highly associated with the body colour patterns in the black-dotted nose of Hanwoo. The importance of the purebred as genetic resources has been highly emphasized. The Ministry of Agriculture of Korea has thoroughly investigated the genetic resources of the domestic livestock and established the gene bank of the Korea native cattle's in the year of 2004. Recently, the molecular researches including the Next Generation Sequencing (NGS) are very actively being undertaken. However, the phenotypic and genetic characterizations of hair colours, colour patterns, and nose colour patterns in the Korea native cattle's are not clearly identified yet. Up to now, the most common methodology to identify the hair colour patterns for cattle's is the use of MC1R (melanocortin 1 receptor) marker in that they identify the function of phaomelanin or eumelanin associated with the colour patterns of

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cattle (Coneet al., 1996; Lee et al., 2002). Even though this methodology also was supported by other reports (Jackson, 1993; Robbins et al., 1993), the use of MC1R is yet not clear enough to identify the true colour patterns of Hanwoo. For the studies of genetic characteristics with sequence differences by random marker analysis even within a breed, methods of NGS (Eck et al., 2009), SNP (Kawahara-Miki et al., 2011), and RAPD (Cornuet et al., 1999) have been widely practiced to identify the genetic diversity and similarity. The use of RAPD-PCR has also been widely used since 1990's and is also being favourably and widely applied to evaluate the variation of a specified gene specification. Kim et al. (2011) studied the specific patterns of the Blacked-dotted nose of Hanwoo by RAPD-PCR to investigate the difference between the Blacked-dotted nose and the normal yellow nose in Hanwoo. In this study, we investigated the genetic diversity and similarity between the Black-dotted nose and the normal yellow nose in Hanwoo by analyzing the specific gene variations expressed in them.

Material and Methods

Sample selection and extraction of genomic DNA

Five Hanwoo with black-dotted nose and 5 Hanwoo without black-dotted nose (normal yellow nose) were selected from farms located in Ansung, Gyeongg-Do Korea. The sampling was taken after they were slaughtered Na et al. (1986) and Lee et al. (2011), based on the expression patterns of black-dotted nose. Each cattle was identified with the registration number proven by RDA (Rural Development Administration), Korea and then, the pieces of nose skin were taken and stored in the liquid nitrogen tank at -196°C. The 250 mg of nose skin piece for each cattle was crushed in the liquid nitrogen, stored in the 1.5ml tube, and DNAs were extracted by Invitrogen Easy DNA kit (Invitrogen, CA, USA). RNAs were removed by adding 40 ug/ml of RNase and were left for 30 min at 4°C. Finally, 500 ng/ul of DNA was obtained by the nano-spectrometer with OD 260/280 within the range of 1.9 to 2.0.

RAPD-PCR analysis for the Marker

The choice of primer for PCR was taken by the 5 sets of URP markers (JK, Anseong, KOR) which contained GC contents over 60% of the total sequence from RAPD analysis. For PCR amplification, 25 ul of PCR preparation solution, 100 ng/ul of DNAs mixed with 2 ul of 10×reaction buffer, 2.5 mM of dNTPs, 10 pmol of URP primer, and 2 unit Taq DNA polymerase were used and finally, distilled water was added. After denaturation for 5 min at 95°C following with denaturation for 1 min at 94°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C, which was repeated 40 times, the final extension was conducted for 8 min at 72°C and then, finally stopped at 4°C. To check the consistence of the experiment, the experiment was repeated 3 times to confirm the same results.

Electrophoresis and genetic diversity analysis

To see the polymorphic patterns of the gene, the electrophoresis was operated for 1 hr at 100 V on 3% agarose gel and then, was identified under UV transilluminator (UVP-UK). The identification was done by checking with the standard Molecular Weight (MW). For phylogenic analysis, all the samples were analyzed by AlphaEase FC (Alpha Innotech, San Leandro, CA, USA) and MEGA5.05 following the method of Khatun et al. (2012) and Tamura et al. (2004, 2011). The dendrogram was drawn by the unwighted pair group method with arithmetic averages (UPGMA) using MEGA5.05 after the data were coded 1 for banding and 0 for no banding. The genetic distance was computed by the method of Nei and Li. (1979); dxy=1-(2nxy/(nx+ny), where nx and ny= no. of bands; and, 2nxy= no. of common bands between the individuals.

Results

Analysis of genetic diversity and similarity using a RAPD marker for black-dotted nose in Hanwoo

The black-dotted nose and normal yellow-coloured nose (the most common type of Hanwoo in Korea) were compared for their genetic diversity and similarity using the 5 RAPD primers with the GC content over 60% following the method suggested by Kim et al. (2011). The unique band patterns for genetic diversity and similarity were found from 150 bp to 2000 bp and significant no. of band markers was 227 among which 75bands (33.1%) were monomorphic and 152 bands (66.9%) were polymorphic (Fig. 1). From the analysis of RAPD primers, the percentages of polymorphisms were 73.7% for URP01, 58.6% for URP02, 81.6% for URP03, 61.9% for URP04, and 33.3% for URP05, correspondingly. It was found that URP03 yielded the highest frequency of 73.7% (Table 1).

Genetic distance among individuals

Through the analysis of genetic diversity using the method of Nei and Li (1979), the larger score indicates the further similarity and the smaller, the closer similarity (Table 2). There was no significant difference among the black-dotted nose but in comparison with BDnHW5 vs. BDnHW4 and BDnHW3, the distances were 0.111 and 0.136, respectively. For other black-dotted nose in Hanwoo, the genetic distances were found in the range of 0.026 to 0.084. For no black-dotted nose of Hanwoo (the yellow-coloured nose), the genetic distances were in the range of 0.012 to 0.089. Exceptionally, the distance within the normal yellow-coloured nose between NnHW1 and NnHW4 was found 0.247. Overall, the genetic distance between the black-dotted and the normal yellow-

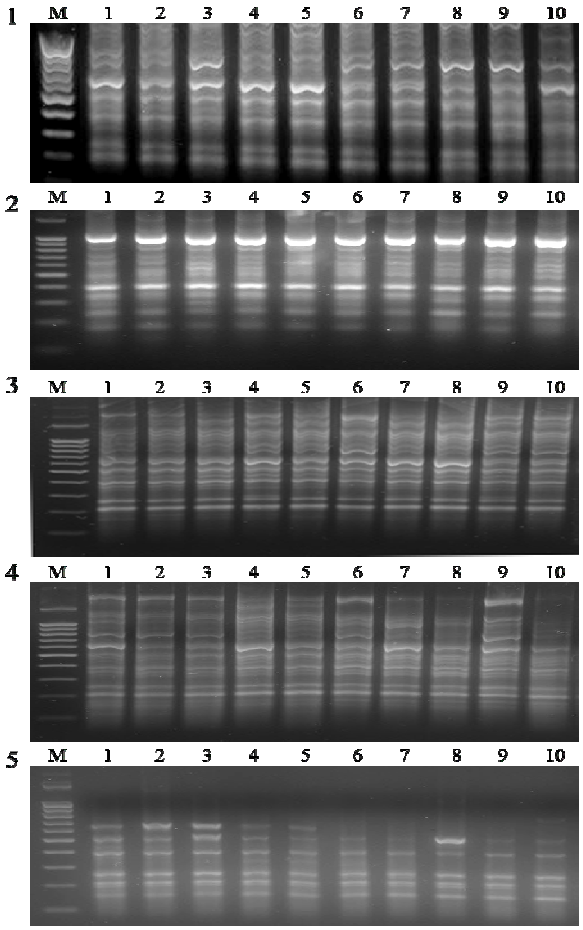

Fig. 1: RAPD profiles using URP primer in the yellow-coloured nose of Hanwoo and black-dotted nose in Hanwoo. The number corresponds to the serial of the genotypes. M : 100bp DNA ladder, lane 1-5 : Black dot nose cattle, lane 6-10 : Yellow nose cattle.

coloured nose in Hanwoo ranged from 0.000 to 0.256, which was not significant but showed some indications of differences.

Dendogram Analysis (5 vs. 5)

Polymorphic band patterns of the DNA as genetic markers were used to detect the genetic similarity between the black dotted nose and the normal yellow-coloured nose in Hanwoo using the UPGMA by MEGA software. The result was given in Fig. 2. For the dendrogram analysis among the 10 cattle's of Hanwoo (5 of the black dotted and 5 of the normal yellow-coloured nose), 2 main clusters of (I) and (II) were grouped and within each main cluster, sub-clusters of IA (1) and IA (2), IB (1) and IB (2) were re-divided. For the main cluster of (I), the 5 black-dotted noise Hanwoo were all included and 3 of the normal yellow-coloured nose were included. For the main cluster of (II), 2 of the normal yellow-coloured nose were included, which implies that

Table 1: List of detected on the monomorphic and polymorphic bands

Primer code	band	No. of monomorphic bands	No. of polymorphic bands	Polymorphism (%)
URP-01	38	10	28	73.7
URP-02	35	18	17	58.6
URP-03	38	7	31	81.6
URP-04	42	16	26	61.9
URP-05	30	20	10	33.3
Total=5	227	75	152	66.9

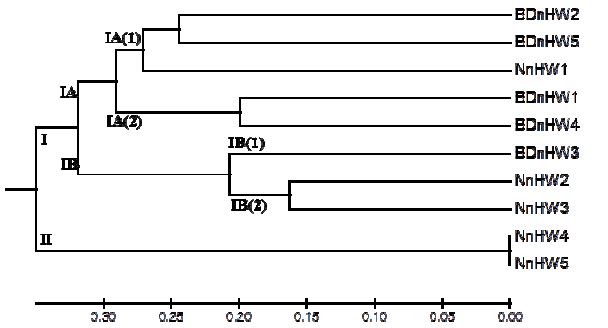

Fig. 2: The unweighted pair group method of analysis (UPGMA) dendrogram based on summarized data regarding differentiation among cattle.

genetic inheritance for the colour patterns on nose for Hanwoo was assumed to follow well the mendelian segregation. From the analysis of dendrogram, we found that there was a genetic difference between the black-dotted nose and the normal yellow-coloured nose in Hanwoo.

Discussion

For the studies of expression for the black-dotted nose in Hanwoo, Lee et al. (2002) reported that the expression of the black-dotted nose could determine the body colour patterns especially in Chik-So (One of the Korea native cattle's with stripped colour patterns on body). And also, Lee et al. (2012) indicated that the colour richness on nose is highly associated with the hair colour and colour patterns on body. The Hanwoo with black-dotted nose used in our study are officially registered as purebred. The purebred Hanwoo does not show black dots but solid yellow or brownish-yellow colour on nose. The expression of different colour patterns on nose in cattle could be highly associated with the hair colour patterns on body. However, the colour expression could be different in different breeds of cattle's. In the study of Park et al. (2012), proven sires of Hanwoo and their 816 progeny which were proven for their parentage in 9 different regions were sampled. Among them, the different colour patterns on body were 6.25 % and the frequency of the black-dotted nose was 65.81 %. However, no significant difference was detected

Table 2: Estimation of pairwise genetic distance between experimental cattle

Tag No.	BDnHW1	BDnHW2	BDnHW3	BDnHW4	BDnHW5	NnHW1	NnHW2	NnHW3	NnHW4	NnHW5
1	***									
2	0.059	***								
3	0.011	0.048	***							
4	0.053	0.111	0.064	***						
5	0.084	0.026	0.073	0.136	***					
6	0.023	0.036	0.011	0.075	0.062	***				
7	0.047	0.012	0.035	0.099	0.038	0.024	***			
8	0.111	0.053	0.100	0.163	0.027	0.089	0.065	***		
9	0.034	0.024	0.256	0.087	0.050	0.247	0.012	0.077	***	
10	0.059	0.000	0.048	0.111	0.026	0.036	0.012	0.053	0.024	***

BDnHW: Black-Dotted Nose in Hanwoo, NnHW: Normal nose Hanwoo (yellow-coloured nose of Hanwoo).

between the black-dotted nose and body colour patterns. The expression of the black-dotted nose is more frequent than the different colour patterns on body, which is possibly due to the variants of MC1R (melanocortin 1 receptor) that is linked to the colour deposition (Lee et al., 2002). And also, melanocortin 1 receptor (MC1R) gene (Klungland et al., 1995; Rouzaud et al., 2000), tyrosinase (TYR), tyrosinase-related protein (TRP-1), dopachrome tautomerase (DCT), and genes associated with the synthesis of melanin (Guibert et al., 2004; Wang and Hebert., 2006) were reported to be associated with colour patterns on body in cattle's. Some studies (Giradot et al., 2005& 2006; Royo et al., 2005) also supported that variants in base sequences of the agouti gene and colour patterns are associated each other. However, the black-dotted nose in Hanwoo has not been clearly defined yet. From our study of genetic diversity and similarity between the black-dotted nose and the normal yellow-coloured nose in Hanwoo, the monomorphic band pattern shown was 33.1% and the polymorphic bands were 66.9%. The highest genetic distance between them was estimated 0.256 and in the dendrogram analysis, among IA and IB within cluster I, most of the black-dotted noses were in IA, indicating the expression of the black-dotted nose could be due to mutation and the ancestral genetic crossing. Thus, this genetic distance can be useful as the differential genomic information in the normal nose and black dots nose of Hanwoo.

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