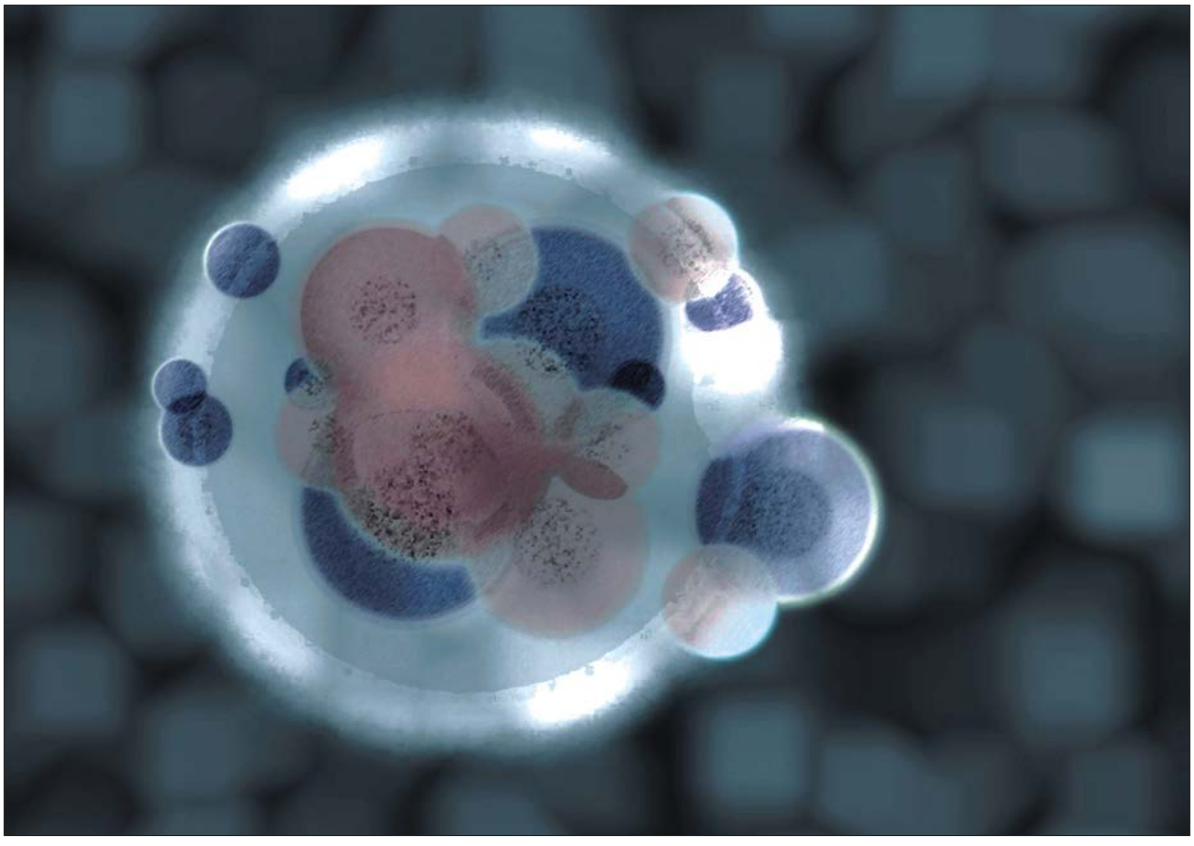

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Genetic Similarity by RAPD Between Pure Lines of Chickens

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Abstract: Twelve primers were used to characterize the genetic similarity among ten chickens which named RIR I, RIR II, Barred I, Barred II, Colombian Rock, Line-54, Blue Line, Maroon Line, Black Line and Brown Line provided by Ankara Poultry Research Institute-Turkey. The objective of this study were to determine PCR-based genetic similarity between lines. Totally, 35 bands were scored for the dendogram prepared on the bases of a similarity matrix using the UPGMA algorithma. The highest and lowest genetic similarities were found to be 0.3773 between Bar I and Maroon and 0.0899 between Col and Bar II, respectively.

Key words: Chicken, RAPD, genetic similarity, pure line, dendogram

INTRODUCTION

RAPD is a technique enabling to detect genetic variation and genetic distance[1]. Up to date, they were common used for the genetic mapping and the selection criteria of characters in poultry[2,3]. In poultry, RAPD methods have been used to detect specific markers[4], genetic similarity between poultry species[5,6] in chicken[7,8], in quail[9] and genetic mapping[10]. Smith *et al.*[6] showed that by using 60 RAPD primers in chicken and turkey populations, genetic relatedness between populations is high but between species are low. Wei *et al.*[11] used the RAPD markers for the genetic characterization of well-developed lines and total 22 polymorphic bands of 120 markers were found and two of them were seen to be related to sex. In this study, using the markers, the similarity of the inbred lines will be estimated.

MATERIALS AND METHODS

Blood samples: The blood samples of chicken lines (brown egg color) RIR I, RIR II, Barred I, Barred II, Colombian Rock, Line-54, (white egg color) Blue Line, Maroon Line, Black Line and Brown Line were collected from Poultry Research Station, Ankara, Turkey.

DNA extraction: DNA extraction was handled by method of Sharma *et al.*[9] DNA amount and its purity were tested using a spectrophotometer based on 260 and 280 nm absorbance.

Primers and PCR: In the study, 12 primers screened as seen in Table 1. These PCR primers designed using published chicken gene sequence data (usually cDNA) to amplify segments of the gene's cDNA for use in RT-PCR or other gene expression assays (Generally these do not amplify well using genomic DNA as template due to introns which separate primer binding sites.) Supplies of each primer pairs are limited; up to 20-40 primer pairs of choice distributed (http://poultry.mph.msu.edu/resources/Resources.htm). PCR mixture (25 μL) consisted of 0.64 U taq DNA polymerase, 25 pmol dNTPs, 25 pmol primer, 50 ng template DNA and was placed on a thermal cycler (Hybaid) which carries a programme included initial step 94°C for 2 mins followed by 35 cycles with 94°C 1 min, 55-48°C 1 min, 72°C 1 min and final step 72°C 10 min. The PCR products were runned on 2% agarose gel stained with ethidium bromide (1%, 100 μL). The RAPD band pattern was displayed on a UV transilluminator and photographed.

Table 1: The sequence of the primers used and their annealing temperature

Genbank No	Primer	Sequence 5'-3'	Annealing time (sec)
63382	ETS 1-2	GGCAGGGCGGCGGGGTAGT	55
464146	HOX 7-1	CGATGGGCGGCGAGGAGGAG	55
558575	MYB -2	GCCGGGACATGCCAATAGA	48
2655421	CDC 37-1	CAGGCCCGCGTGGAGAGGATGGA	55
2209150	BKJ-1	TGCGATCCAGCCCCCACCAG	55
2369862	SPI 1-2	CCCCCTTCCCATCACCTCA	55
558575	MYB-1	GTGGTGGCTGGGAACAAACTGAG	55
2369869	SPI 1-1	CCTCATTCCCCCTCCCTCTG	55
1399186	ZFP 161-2	GCAGGAACCGCAGACAAAA	48
2745888	AKT-1-2	ACAAAGTGCGTGGAAATCTAATCT	55
2745888	AKT 1-1	CCGGACGGTATTATGCTATGAA	48
2623878	HSPE 1-2	GAATGTTCACCGTCTCTAAA	48

Data analysis: The RAPD bands were scored for their presence (1) or absence (0). The similarity index between each two lines was analysed by Nei and Li[12] and a dendogram was made using NTSys UPMGA computer programme.

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Table 2: The size and number of bands used in the study

Primer	300	350	400	450	500	600	650	750	900	1000	1400	1500	2000	No. bands
ETS 1-2			v		v	v		v						4
HOX 7-1					v					v	v			3
MYB -2	v		v											2
CDC 37-1										v		v		2
BKJ-1					v	v	v		v	v			v	6
SPI 1-2		v			v									2
MYB-1			v					v		v	v			4
SPI 1-1	v	v		v				v		v	v			6
ZFP 161-2			v		v	v		v				v	v	6

RESULTS AND DISCUSSION

Among twelve primers, nine of them amplified successfully genomic DNA from ten samples of egg-producing chicken lines which six of them have brown color lines and four of them have white color lines as seen in Table 2. Amplified nine primers gave totally 35 bands in chicken genotypes (Fig. 1) which show 42% polymorphism. At the similar study, Singh and Sharma[13] used 12 primers in white leghorn and found 22% polymorphism which sourced from high homology between genotypes. The size and number of bands are given in Table 2. The highest number of bands was seen in BKJ-1, SPI-1 and ZFP 161-2 primers. The lines used in this study are egg-producing chicken lines and average genetic similarity was found to be between 8.9 and 37.73%.

RAPD markers are effective to detect similarity between chicken lines and is potential tool for studying genetic relationships[1,11,14,15] and the dendograms of the similarity matrix of chicken lines with their pictures was seen in Fig. 2.

Two primers ZFP161-2 ve BKJ-1 had very specific bands. ZFP 161-2 produced a band which was seen only the genotypes of Barred I with the size of 1500 bp. Also,

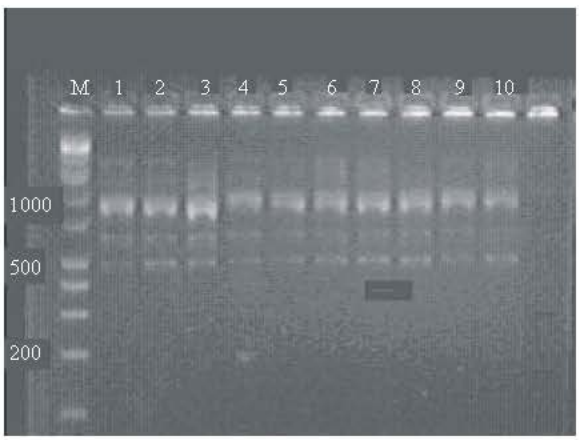

Fig. 1: RAPD amplification products generated by primer BKJ-1

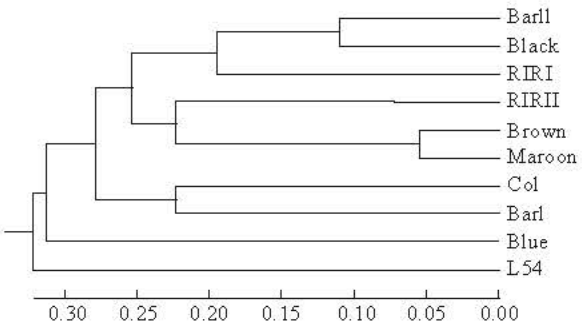

Fig. 2: The dendogram of chicken lines

BKJ-1 had another specific band that has a size of 2000 bp seen in the genotypes had brown egg. However, it was not seen expected results from the angle of egg color genetic as brown and white egg. The number of bands used in this study is lower when comparing other studies[13]. The reason of the number of low band is consider to be long sequence primers used in this study[6,13].

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