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Effectiveness of RAPD Marker in Generating Polymorphism in Different Chicken Population

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Abstract: A study was conducted to know the effectiveness of Randomly Amplified Polymorphic DNA (RAPD) marker in generating polymorphism in different chicken populations. Four of the twenty random primer screened yielded distinct RAPD profiles. Among 39 fragments amplified from these 4 primers, 25 of them showed polymorphism. The average number of amplified bands per primer ranged from 9 to 11. The study revealed that RAPD markers were effective in detecting polymorphism in different chicken breeds. However, comparatively large numbers of random primers were required to detect satisfactory polymorphism in different chicken populations.

Key words: RAPD marker, chicken, polymorphism

INTRODUCTION

The development of DNA-based markers has had a revolutionary impact on gene mapping and more generally, on all of animal and plant genetics[1]. With DNA-based markers, it is theoretically possible to exploit the entire diversity in DNA sequence that exists in genome.

The most commonly used DNA-based markers include southern hybridization-based (minisatellite/ microsatellite) and PCR-based (arbitrary primer-PCR or randomly amplified polymorphic DNA-PCR). Among available DNA-based marker microsatellite, Restricted Fragment Length Polymorphism (RFLP) markers are the most efficient in generating polymorphism in livestock species[2,3]. These markers, though capable of generating substantial polymorphism, involve a costly, time intensive and laborious assay procedure and biohazardous radioactive elements. Another relatively new type of marker, based on amplification of DNA by polymerase chain reaction using short primers homologous to random annealing sites in genome are Randomly Amplified Polymorphic DNA or RAPD[4]. The RAPDs have several unique advantages such as they do not require the prior knowledge of target sequence, need only small amount of DNA and are simple, fast and less costly[5]. Therefore, the RAPD may provide a highly polymorphic system of choice, capable of generating polymorphism. The RAPD has successfully been used in generating polymorphism in livestock and poultry[6,7]. However, Zhang *et al.*[8] and Wei *et al.*[9] reported that RAPD is less effective in generating polymorphism and require large number of random primer to produce sizeable polymorphisms. Hence in the present study, an attempt has been made to evaluate the effectiveness of RAPD in generating polymorphisms in different chicken populations.

MATERIALS AND METHODS

Populations: The four chicken population viz., Rhode Island Red (RIR), White Leghorn (WL), Commercial egg type strain Shaver 579 (SH) and slow growing broiler strain ISAi757 (ISA) constituted the base material for present study. These chicken populations are differed in origin and breeding history.

Blood collection and DNA isolation: About 500 μL blood from individual birds of each population was collected into 1.5 mL eppendorf tube using the procedure suggested by Hoelzel[10]. The high quality genomic DNA was isolated by phenol-chloroform extraction using the protocol adopted by Alam *et al.*[11] with some modifications. DNA quality was checked by electrophoresis in a minigel and quantified using a spectrophotometer (Spectronic® GENESIS™) based on absorbance at 260 and 280 nm, respectively.

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Primer selection: Initially twenty 10-mer primers from one kit (Kit A) of random sequence (Operon Technologies, Inc., Alameda, California, USA) were screened to test their suitability for amplifying chicken RAPDs that could be accurately scored. Primers were evaluated on the basis of intensity or resolution of bands, repeatability of markers and consistency within individual and potential to differentiate populations (polymorphism). A final subset of four primers (Table 1) out of twenty exhibiting better quality banding patterns were selected unbiasly for the analysis of the whole sample set of the four chicken populations.

PCR amplification: The amplification conditions were based on Williams *et al.*[12] with some modifications. PCR reactions were performed on each DNA sample in a 10 μL reaction mix containing 1 μL of 10x Ampli Taq polymerase buffer, 2 μL of 10 μM primer, 1 μL of 250 μM dNTPs (Takara, Japan), 1 unit of Ampli Taq DNA polymerase (Takara, Japan) and 50 ng of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 34°C and elongation or extension at 72°C for 2 min. After the last cycle, a final step of 7 min at 72°C was added to allow complete extension of all amplified fragments.

Agarose gel electrophoresis: The amplified product from each sample was separated electrophoretically on 1% agarose gel (Nacalai tesque, Inc, KYOTO, Japan) containing ethidium bromide in 1XTAE buffer at 120 V for 1½ h. DNA bands were observed on UV-transilluminator and photographed by a Gel Cam Polaroid camera.

Data analysis: All distinct bands or fragments (RAPD markers) were scored visually on the basis of their presence (1) or absence (0), separately for each chicken for each primer. For more accuracy, two independent persons performed band scoring. Bands or RAPD markers not identified by the two readers were considered as non-scorable. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for comparing the frequencies of all polymorphic RAPD markers among populations with 1000 simulated samples using POPGENE (Version 1.31)[13] computer program.

RESULTS AND DISCUSSION

Among twenty primers (Table 1) initially tested, four primers namely OPA12, OPA16, OPA18 and OPA20 produced comparatively maximum number of high intensity amplification products with minimal smearing and generated 39 distinct bands (RAPD markers), all of which 25 bands (64.10%) were considered as polymorphic (either occurring in or absent in less than 95% of all individuals) among chicken populations (Table 2). The amplification profiles in various chicken populations are presented in Fig. 1. Among the primers, primer OPA20 gave DNA profiles with more bands than OPA18, OPA16 and OPA12, respectively. The chicken breeds seem to differ for the average number of bands per primer, ranging from 9 to 11. Since the amplification from these arbitrary sequenced primers depends upon the presence or absence of annealing site on template DNA, different

Table 1: Parameters of the Operon random primers used for screening four chicken populations

Primer codes	Nucleotide length	Sequence (5' to 3')	(G+C) %
OPA01	10-mer	CAGGCCCTTC	70.0
OPA02	10-mer	TGCCGAGCTG	70.0
OPA03	10-mer	AGTCAGCCAC	60.0
OPA04	10-mer	AATCGGGCTG	60.0
OPA05	10-mer	AGGGGTCTTG	60.0
OPA06	10-mer	GGTCCCTGAC	70.0
OPA07	10-mer	GAAACGGGTG	60.0
OPA08	10-mer	GTGACGTAGG	60.0
OPA09	10-mer	GGGTAACGCC	70.0
OPA10	10-mer	GTGATCGCAG	60.0
OPA11	10-mer	CAATCGCCGT	60.0
OPA12*	10-mer	TCGGCGATAG	60.0
OPA13	10-mer	CAGCACCCAC	70.0
OPA14	10-mer	TCTGTGCTGG	60.0
OPA15	10-mer	TTCCGAACCC	60.0
OPA16*	10-mer	AGCCAGCGAA	60.0
OPA17	10-mer	GACCGCTTGT	60.0
OPA18*	10-mer	AGGTGACCGT	60.0
OPA19	10-mer	CAAACGTCGG	60.0
OPA20*	10-mer	GTTGCGATCC	60.0

*Primer showed substantial polymorphism

Table 2: Range of distinct genomic DNA bands and polymorphic bands amplified by the RAPD-PCR technique

Primers	No. of band amplified	Polymorphic band
OPA-12	9	6
OPA-16	9	6
OPA-18	10	7
OPA-20	11	6
Total	39	25

Table 3: Number and percentage of polymorphic loci across primers in four chicken populations

	Primers							
	OPA12		OPA16		OPA18		OPA20	
Populations	No.	%	No.	%	No.	%	No.	%
RIR	5	55.55	4	44.44	4	40.00	6	54.55
WL	6	66.66	6	66.66	3	30.00	3	27.27
SH	3	33.33	2	22.22	4	44.44	3	27.27
ISA	4	44.44	4	44.44	0	0	5	45.45

Table 4: Total number and percentage of polymorphic loci of four chicken populations

Populations	No. of polymorphic loci	Percentage of polymorphic loci
RIR	19	48.72
WL	18	46.15
SH	12	30.77
ISA	13	33.33

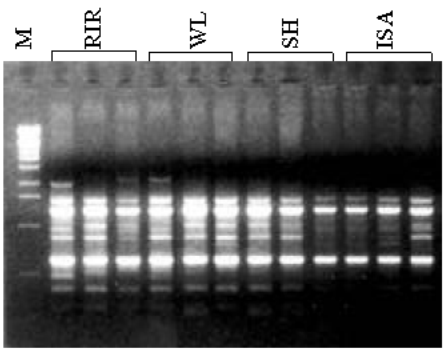

Fig. 1: RAPD profile generated using OPA12 primer form four chicken populations. M: Molecular weight marker

primers are expected to give different numbers of amplicons. The results agreed well with the findings of several authors[6,14-16]. They concluded that the number of bands amplified differed with the primers.

The proportion of the primers capable of detecting the polymorphism among the breeds evaluated depends upon the genetic background of the breeds, genetic distances between the breeds and complexity of the genome. In present study, only 4 primers out of 20 could detect polymorphisms among the chicken breeds. Earlier reports also showed 4 to 13% proportion of polymorphic primers[6,17]. The overall polymorphism produced by the primer OPA20 was the lowest while that produced by primer OPA12 was the highest (Table 3). From Table 4 it was revealed that the highest percentage of polymorphic loci was found in case of RIR chicken and the lowest percentage of polymorphic loci was found in Shaver 579 chicken.

In conclusion, the present study revealed that RAPD markers are effective in detecting polymorphism in different chicken breeds. However, comparatively large numbers of random primers are required to detect satisfactory polymorphism to be utilized for genome analysis.

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